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PRACTICAL MICROSCOPY

PRACTICAL MICROSCOPY

AN INTRODUCTION TO MICROSCOPICAL METHODS

BY

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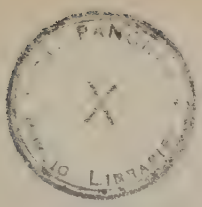

PREFACE TO THE THIRD EDITION

In preparing a third edition of 'Practical Microscopy,' first published in 1905 under the title of 'Elementary Microscopy,' and which, owing to other demands upon the author's time, has been for some time out of print, the second, third, fourth, and fifth chapters have, of necessity, required much alteration. These chapters have been written with a view to bringing under the notice of workers apparatus which may be of service to them in their work, or to discussing the value of others which they may be thinking of purchasing. Chapter VI., dealing with the Practical Optics of the Microscope, still contains no more theory than is necessary to understand the subject, and, like the succeeding chapter on the Manipulation of the Microscope, endeavours to provide that practical information and advice which experience has shown the author to be most needed.

Matters which periodically come prominently forward with regard to high magnifications and their value, the limits of vision and of resolution, photo-micrography with ultra-violet light, and apparatus for examining ultra-microscopic particles, were fully dealt with in the previous edition, and need no alteration except to add a few paragraphs with regard to further developments in this direction. A chapter on Photo-micrography is again included, and the chapter on Microscopical Technique, which replaced the earlier one on Mounting, has been retained, as in it the author,

without attempting the impossible task of describing special methods, has endeavoured to give a practical explanation of the broad principles of preparation, staining, etc., generally so little understood, and to leave their applications in zoology, botany, human histology, or pathology to be sought for elsewhere. The pages on section-cutting, which deal with this subject at considerable length, have also been retained unaltered in order to assist as much as possible the comparatively inexperienced worker, for whom the book is mainly written.

As before, the author wishes to acknowledge his indebtedness (like all other workers) to Mr. Bolles Lee's 'Microtomist's Vade-Mecum,' to Professor Guyer's 'Animal Micrology,' and to Mr. Conrad Beck's Cantor Lectures on the microscope, from which he obtained several useful suggestions. He wishes also to put on record here his grateful appreciation of the secretarial work in connection with this book, and at all times and in all his labours so willingly and efficiently rendered to him by his elder daughter. Thanks are again due to many firms of opticians for their ready courtesy in lending many of the illustrations, several of which have been specially made. The other illustrations are drawn by the author.

Redcourt, Adams Road,
Cambridge,
January, 1926.

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PRACTICAL MICROSCOPY

CHAPTER I

INTRODUCTORY

In few things is a little initial advice and help more valuable than when one is first taking up the use of an instrument like the microscope. There are many who are desirous of purchasing a microscope, and then learning how to use it, who feel bewildered by the multiplicity of stands and accessories advertised by the various English and Continental makers. There are others who already possess microscopes, but have not yet mastered even the principles of an instrument which is essentially one of precision, and so fail to use their microscopes to advantage, with the result that they soon relegate them to comparative obscurity when the first novelty of possession has departed. There are yet others who use the microscope in scientific work, but even they have not mastered these principles, and scarcely realize how much their work might be facilitated by doing so. Several excellent manuals are to be obtained on the subject, but they deal for the most part with more advanced microscopy and with its theory, whilst the keen competition amongst our leading opticians, and the constant change and progress to which it gives rise, render a not inconsiderable portion of such books quickly out of date. The aim of the present work is first to indicate to the beginner, or to the student, how he may most profitably spend his money on the necessary outfit—what, in fact, are the essential requirements he must look for in his instrument, together with

its accessories—and then to explain in detail its manipulation and management, so that he may learn to use his instrument and its accessories to the very best advantage. Theory will not be more fully entered into than is necessary for explanatory purposes, the aim of the book being essentially practical, and whilst an attempt is made to give instruction on the elementary and broad principles underlying modern methods of preparing objects for the microscope, it has been decided that Nature, as revealed by the microscope, or technical applications, are quite outside the scope of this book. The suggestions as to the choice of a microscope are not directed to providing an outfit suitable only for whiling away a pleasant hour or two—for 'looking at pictures,' in fact. Instead, it is the aim of this book so to advise the beginner that afterwards, when he has gained experience for himself, he may feel that what he has bought is not only capable of showing him the infinitely little in Nature as a pastime or pursuit, but will be equally serviceable for real and serious work in medicine, in zoology, in botany, or any other of the countless ways in which the modern microscope has become a necessity, and will enable him to use the microscope for that purpose in the best possible way. In spite of the universal use of the microscope in our science laboratories and medical schools, little practical instruction in the use of the instrument is generally given, and the author's experience has been that there are large numbers of men who are only too glad to obtain the instruction which the following chapters endeavour to provide.

CHAPTER II

THE SIMPLE AND COMPOUND MICROSCOPE

The Simple Microscope.—Strictly speaking, a microscope may be either **simple** or **compound.** By a simple microscope is understood a lens, or combination of lenses, which magnifies the image but once, and which is generally either held in the hand or provided with some simple form of support. Of these, the best known are the ordinary reading-glass, made in all sizes, and the small **pocket magnifiers,** mounted in vulcanite or imitation tortoise-

FIG. 1.—EBONITE POCKET MAGNIFIERS.

shell, containing one or more lenses (Fig. 1). All these are of the simple double-convex type, and, though cheap, have the drawbacks and imperfections incidental to such a type. These drawbacks include much distortion of the image towards the edges of the field of view, and its further disturbance by fringes of colour. The details also are not as sharply shown as they should be. These imperfections may in the first place only be noticed when the lens is compared with one of a higher type, such as those about to be mentioned, but if the lens is used for prolonged work, supported by a stand, the resulting

fatigue to the eye becomes very marked. These magnifiers may be obtained from almost any optician at prices ranging from one shilling upwards. The watchmaker's eye-glass is of this kind.*

Linen Tester.—The **Linen Tester** is of the same description, but is made with a folding support, at the bottom of which is a small square opening in which to count the number of threads to the inch. Another form of simple double-convex lens is mounted on three legs, and the focus is adjusted by means of a screw. This last is sometimes made of two lenses with a diaphragm between.

Coddington Lens.—The **Coddington Lens** is a single double-convex lens which has a groove cut all round the middle, this groove being blackened or filled with black cement so as to act as a limiting diaphragm, thus cutting out the peripheral aberrations inherent in such a lens. The lens itself is of considerable thickness, being really only the central portion of a glass sphere. The performance is very imperfect, and it is now less frequently met with than formerly.

Stanhope Lens.—The **Stanhope Lens** is also a double-convex lens, but the curvatures are unequal. In use, the object to be examined is placed on the side with the least convexity, and in actual contact with it, and it is just in focus when the other side of the lens is turned towards the eye. It is obvious that its uses are very limited.

* The author remembers that a good many years ago an ingenious street-vendor used to sell in the neighbourhood of St. Paul's Churchyard a microscope of this sort made out of a pill-box, with a small hole in the lid, in which was a drop of clear balsam. A similar, but of course temporary, microscope can be made by boring a hole in a blackened card with a red-hot needle, and inserting therein a drop of water.

Aplanatic Magnifiers.—Of late years an entirely new form of simple hand-lens has come largely into use, and few working microscopists are without one or more of them, either for pocket use or for dissecting. These are achromatic triplets, which are also aplanatic. They are often spoken of as 'Steinheil lupes,' or sometimes as 'platyscopic' lenses. There are several formulæ

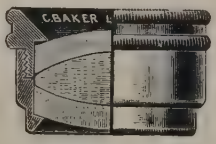


FIG. 2.—STEINHEIL LUPE (SECTION).

adopted by various makers, and there is not much to choose between them, but their construction is as illustrated (Fig. 2), and their distinctive features are good definition, freedom from colour, a flat field by

FIG. 3.—APLANATIC MAGNIFIERS.

means of which the whole of the lens can be utilized, the image remaining sharply in focus up to the very margin, and ample working distance. In the higher powers particularly they have therefore a marked advantage over the simpler form of lens. They are supplied by all the principal microscope makers in magnifications of 6, 10, 15, and 20 diameters, mounted

in ebonite or German silver, either in a simple ring to fit into a dissecting holder, or in a mount for the pocket (Fig. 3). Only lenses made by well-known makers should be selected, the most convenient power for everyday work being perhaps 6, and after that 10. For a pair 6 and 15 would be handy sizes, but the working distance of the latter will be found to be rather short. In use, they are held close to the eye.

The Compound Microscope.—By a **compound** microscope is understood a combination so arranged that the image given by the first lens is itself magnified again before reaching the eye. These lenses are called respectively the objective and the eyepiece, or ocular. Opaque objects are examined by means of either natural or artificial light reflected from their surfaces, but the great majority of microscopic objects are specially prepared so that they may be viewed by light transmitted through them in order to show their structure. The source of light can be looked at direct, but this would not be convenient in many cases, and therefore beneath the stage holding the object in position is placed a mirror capable of movement in order to reflect the light through the object into the lenses. Many microscopes, especially the cheaper Continental stands, make no further provision than this, but the improvement in microscopic objectives and the use of higher powers has necessitated the interposition between the mirror and the object of another lens or system of lenses with which to condense the light, and even accurately to focus it upon the object. These are therefore the elementary essentials of a modern compound microscope, and the following explanations concern the elaborations of these essentials which precision of working has called forth. The explanation of the principles of microscopical vision and

of microscopical optics will be left until a later chapter, it being desirable to assist the reader to choose his microscope first, and to explain its principles and use to him afterwards.

Parts of a Microscope.—Leaving aside for the moment the discussion of the optical parts of the microscope, it will be observed that the instrument consists of a **foot** or base (Fig. 4); a **stage** to hold the object; a **mirror** to reflect the light; between this and the stage probably a **sub-stage** carrying a **condenser** to condense the light; and a **limb** bearing a **body tube.** Into the lower end of this tube is screwed the **objective,** whilst into the upper end is inserted the **ocular** or **eyepiece,** the tube itself having a **coarse adjustment** for focussing, either by sliding bodily in a sleeve or by rack and pinion, and being further elaborated by a **fine adjustment** for use with high powers. We shall take all these in turn, explaining them as we proceed.

The Foot.—On the **Foot** depends the stability of the stand as a whole. There are practically two forms—the **pillar and horseshoe** form and the English **tripod.** The advantage of the former is that it is generally a little cheaper, and the sub-stage apparatus is more conveniently manipulated when the microscope is used in an upright position, as is often necessary with medical and zoological workers. In any other than the upright position it is much less steady than the tripod form, whilst for horizontal work, especially for photo-micrography, it is quite top-heavy. The additional weight put into this form of stand to remedy the instability is also a disadvantage. The importance of these points can only be fully appreciated and verified by the practical worker, but the tripod foot, either in its ordinary or modified form of bent claw (Fig. 10, p. 32),

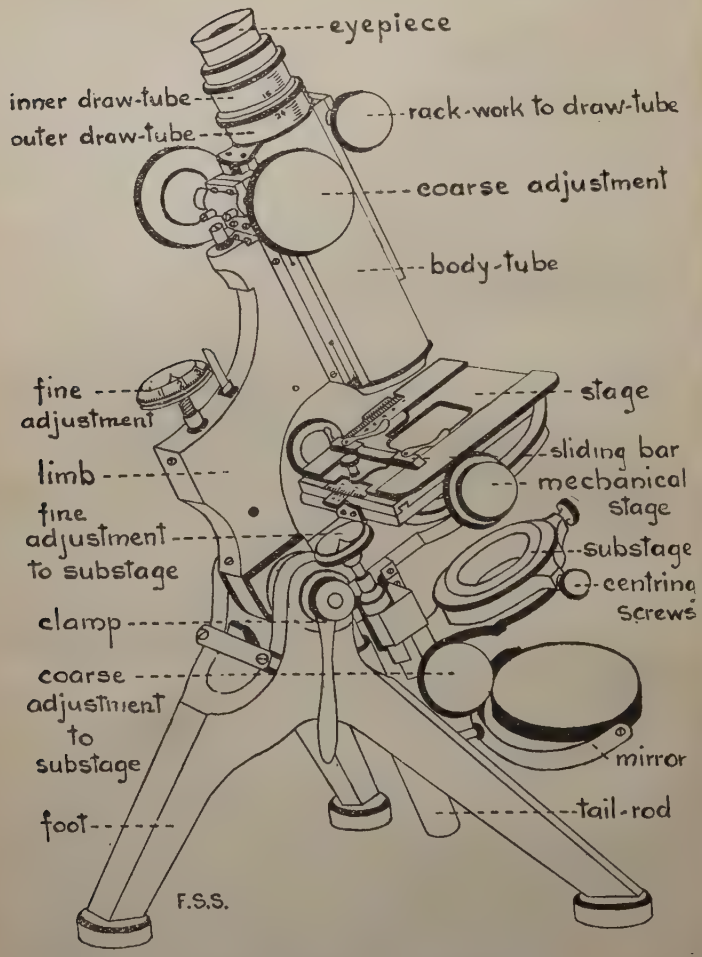


FIG. 4.—PARTS OF A MICROSCOPE.

has come more and more into favour—in England, at any rate—and we unhesitatingly recommend its choice. At the same time, the pillar and horseshoe or claw foot is almost universal on the Continent, and American makers have borrowed the same model. As a result most of our own makers have been obliged to follow suit, at any rate in their cheaper models. Whatever form of stand be adopted, ascertain that the actual points of contact with the table are not more than three, or a most irritating unsteadiness will result.

The stand must be capable of inclination. It is only the cheapest form of stand for rough laboratory use that is without means of inclination.

The Stage.—The **Stage** should be large and rigid. In its simplest form it will be provided only with two springs to hold the slide in position. These should be adjusted so as not to press too strongly upon the slide, otherwise the fingers will be unable to make the necessary delicate alterations in the position of the object. The surface may be of brass, of vulcanite, or of glass; for ordinary purposes vulcanite is preferable. The aperture should be large, not less than an inch in diameter, and preferably considerably more. By having a large aperture the end of the finger can be inserted beneath the slide so as gently to raise it when focussing with high powers, the slide being lowered again when the approximate focus is found, and the focus of the objective finally adjusted. By this means the practised microscopist guards against any risk of damaging either his slide or his objective. Mr. E. M. Nelson, who has done so much for English microscopy, has suggested that the opening shall be cut away right to the front of the stage so as to make a horseshoe opening, and the suggestion has been adopted in more than one stand.

Sliding-Bar.—A great improvement upon the ordinary stage springs is a **sliding-bar or carrier,** which slips up and down either in grooves in the stage or outside its parallel edges (Fig. 5). It carries the usual springs, which can be turned aside or brought into use as required. By this means the adjustment of the slide can be carried out with greater precision, and in particular it can be systematically examined by moving alternately the slide horizontally and the bar vertically on the stage.

Mechanical Stage.—In a first-class instrument a further refinement called a **mechanical stage** is necessary

FIG. 5.—SLIDING-BAR TO STAGE, WITH FINDER.

(Fig. 4), though for ordinary work the fingers can be trained to a delicacy of adjustment that will cover most requirements. The mechanical stage has two milled heads which give rectilinear movements horizontally and vertically respectively. The milled heads should preferably be so arranged as not to travel with the stage, otherwise the screws are apt to foul the condenser. The largest and most elaborate stands have a fixed mechanical stage, which may or may not carry a sliding-bar in addition, but all the leading makers have now introduced detachable mechanical stages which

can in many cases be added as accessories after the purchase of the instrument. The detachable mechanical stage, in spite of its occasional advantages, is, however, generally less solidly constructed than the fixed type.

Graduations to Stage.—Yet another refinement is the addition of **graduations** to the stage, both horizontal and vertical, by means of which the position of a slide can be noted for future reference, as it is only necessary to replace the slide in the same position to insure a ready finding again of the same field of view. Such graduations are shown in Fig. 5 as a simple and inexpensive but most useful addition to a stage fitted with a sliding-bar (see p. 10). The graduations are generally ruled off in millimetres, and numbered from 0 to 20 or 30. Where they are marked on a mechanical stage a stop is provided, removable or otherwise, against which to place the corner of the glass slide, and the almost universal adoption now of slides measuring 3 inches by 1 inch renders this easily practicable. In addition, verniers for more exact reading are provided, and by this means rough micrometric measurements can be made in conjunction with a line across the centre of the eyepiece. Other methods will be alluded to when we come to micrometric measurements (pp. 111 and 193).

Rotary Stage.—Microscopes for special purposes, such as petrology, have also a complete rotation to the stage, and the most elaborate form of instrument is provided not only with this, but with a couple of milled heads just beneath the stage, by means of which the centre of rotation can be made accurately to coincide with the optic axis of the microscope in order to insure an absolutely true rotation. However carefully an objective may be constructed by its maker, it will be found that

the optic axis of the microscope varies slightly with each change of objective, and this not only necessitates re-centring the condenser, and often re-finding the object, but becomes specially important and noticeable in case of rotation of the stage. To correct this, either the stage must be re-centred by means of centring screws provided for the purpose, or the objective itself must be adjusted by means of what is called a **centring nose-piece.** The latter way is cheaper, but naturally lengthens the tube; a point which will be dealt with later.

The last refinement of all is a rack and pinion by which the stage can be slowly rotated when necessary, and this should be capable of being thrown into and out of gear. It is seldom required, except in petrological work. There may also be graduations in degrees around the circumference of a circular stage to show the amount of rotation. In its most perfect form the whole of the stage, including the mechanical part, will rotate bodily, though a rotating top plate is sometimes provided instead, which is less expensive and often of service. A protest should be made against the absurdity of providing a microscope with a complete rotating stage, fitted with centring screws, and with elaborate graduations around its entire periphery, but which, owing to the projecting milled heads of the mechanical stage, is incapable of rotation for more than, say, 300 degrees. The rotary stage is not often fitted to English students' microscopes, and is sometimes carelessly made, so that there is a troublesome amount of ' play ' in it when used with high powers.

Mirror.—The **Mirror** is carried upon a tail-rod, either square or circular, and sometimes with an extra joint in it. Under any circumstances it should be capable of being swung aside. In certain microscopes sufficient

movement is allowed in the tail-rod to enable the mirror to be brought above the stage so as to illuminate an opaque object, and we wonder this is not more frequently met with. For this reason we prefer a circular tail-rod when only one joint is provided, the support of the mirror itself having a corresponding circular clip. The mirror should have two faces, plane and concave; they should be of good size, and be hung in a gimbal so as to give universal movements. The concave mirror having a definite focus, it is necessary that there should be a means of focussing it vertically, and generally this is provided. We have, however, come across microscopes in which the movement was too short to allow proper adjustment of the concave mirror. The best way to test this is to reflect and then focus the light of a lamp upon a piece of white paper placed upon the stage of the microscope, the mirror being moved up and down the rod until the requisite focus is obtained. This focus will, however, strictly speaking, vary somewhat according to the inclination of the mirror, due to an alteration in the inclination of the microscope or height of the lamp.

The plane mirror should preferably be 'parallel worked,' otherwise an annoying double or treble reflection will be observed when the condenser is in focus. This may be obviated in most mirrors by rotating them in their cells, as suggested by Dr. Dallinger, until a certain point is reached, when all the images are superimposed. All mirrors might with advantage be mounted so as to admit of this rotation. The optical reason given for this duplication of the image is that an ordinary mirror is somewhat wedge-shaped, and there are, accordingly, distinct reflections from the glass surface, from the silver surface, and from the silver and air surfaces. To obviate this, certain workers use a rectangular prism

instead of a mirror, but this has the disadvantage of requiring that the prism should be large enough for use with a modern condenser with its wide back lens. It is also, as might be expected, much less convenient to use, and is therefore comparatively seldom met with.

C. Baker and Watson have recently taken advantage of the introduction of stainless steel to use this instead of a glass mirror, and so to obviate double images, as well as breakage, or deterioration of the silvering, especially in hot and damp climates.

The Sub-Stage.—This is now a most important adjunct to the modern microscope. Its office is to carry the condenser and other apparatus. Without such a fitting no microscope stand can be considered complete or capable of doing good work with other than low powers. It is the condenser that makes the modern microscope so enormously superior to its predecessors, and it is second only in importance to the invention of the achromatic objective. Its office, as already intimated, is to gather together, concentrate, and focus the light upon the object from beneath. No microscope should be purchased that has not fittings for such a condenser, or to which the condenser cannot, at any rate, be readily added subsequently. In its simplest form it is merely a ring fitted beneath the stage, and centred with the optic axis of the microscope. The inside diameter of this ring should be of the 'universal' size adopted by the Royal Microscopical Society—*i.e.*, 1½ inches full (1·527 inches or 38·786 millimetres). This gauge is not uniformly observed on the Continent, Leitz retaining one of 30·5 millimetres (1·2 inches), and Zeiss one of 36·8 millimetres (1·45 inches). The condenser, in a suitable mount, slides up and down in this

ring, which is often made to swing aside when not required.

Focussing Sub-Stage.—But as the condenser requires focussing like the objective, a focussing adjustment becomes more than a convenience. This in its cheapest form is provided by means of a left-hand screw and milled head working vertically (Fig. 13, p. 35), which is so arranged as to swing the condenser aside when it is quite out of focus and clear of the stage. But in the best instruments a complete sub-stage with rack and pinion is provided (Fig 4, p. 8), and there may even be added an additional fine adjustment to enable the last delicate adjustments to be made without setting up any disturbing tremor. This is, however, a refinement that is only needed for the most critical work. Further, as the centring of objectives generally varies more or less, as already intimated, it is advisable to have some means of centring the condenser, a matter of no little importance in high-power and critical work.

Centring Sub-Stage.—This is provided by two centring screws working at right angles against a spring, or by proper rectangular movements. It is generally found only in compound sub-stages (Fig. 4). But whether a simple ring or a complete sub-stage be provided, it should be constructed in a workmanlike manner. There should be no tendency to shake, a weakness to which the swing-out and spiral-screw forms are somewhat liable, and the centring should remain the same whether the condenser be screwed or racked up or down. A means of rotating the condenser or other fitting by rack and pinion is sometimes provided, but in few cases is this worth the additional cost.

Diaphragm.—The **Diaphragm** is a means of adjusting the light so as to bear some ratio to the apertures of

the various objectives. In its simplest form it is a circular plate containing apertures of various sizes, set just beneath the stage, so that by rotation each of the openings can be brought central with the opening in the stage. An iris diaphragm is also sometimes fitted in this position. The most unsatisfactory form of all is where one or more rings are provided to fit inside the stage opening. But the widespread use of the condenser has necessitated the placing of the diaphragm immediately beneath it, and in this position the plate of diaphragms has given place to a set of loose stops which can be slipped into a suitable carrier, or to the now familiar iris diaphragm.

Condensers themselves are dealt with in Chapter V.

The Limb.—The **Limb** is the arm carrying the body tube, and on its construction much depends. There are practically three forms now in use—that originally designed by Ross, and known as the bar movement, the solid limb, and the Continental limb. They are so closely bound up with the coarse and fine adjustments that we must consider them under these headings.

Coarse Adjustments.—The **Coarse Adjustment** in its simplest form is simply a broad ring in which the body tube slides. It is manifestly only fitted to the least expensive instruments, though when an efficient fine adjustment is provided in addition it is capable of good work up to a certain point, but as it cannot rotate truly in its ring, it necessarily increases troubles due to decentring. Not the least of its disadvantages is, however, the increased danger of damage to either objective or slide, and we consider a thoroughly well-made rack and pinion coarse adjustment, without fine adjustment, preferable to a sliding coarse adjustment and an additional fine adjustment. Whenever possible, and

certainly in all stands intended for good work, the coarse adjustment should be by rack and pinion. Many years ago Swift and Sons introduced a 'diagonal' rack and pinion, now adopted by all the leading makers, which works with exceeding smoothness, and is less sensitive to wear and consequent 'back-lash' than the older rectangular form. In fact, the fitting of any other than a diagonal rack and pinion at once stamps a microscope as inferior. The bearings of the coarse adjustment, or the slides in which it works, should be 'sprung,' and provided with screws for adjustment for wear and tear, and there should be ample bearing surface. The milled heads should be large.

Fine Adjustments.—The **Fine Adjustment** brings us to a subject upon which much controversy has taken place. It is scarcely necessary to explain that good work with high powers cannot be done without an efficient fine adjustment, and of all parts of the microscope this is the one which has most tendency to deteriorate and become inefficient. The compass and design of this book prevent a full discussion of the various types now in use, and of the improvements and refinements which the ingenuity of various workers has brought forth, but the importance of the subject requires explanation in detail of the main points of the principal types. First of these in point of seniority comes Powell and Lealand's form. The coarse adjustment is a massive bar working vertically within the casting which supports the stage. To the top of this and at right angles is bolted a bar or arm which projects beyond the centre of the stage, and into which is screwed the body tube. Within this horizontal arm is a lever of nearly the same length, which is raised and depressed by means of a screw fitted with a milled head, and placed

just behind the junction of upright bar and arm. The fulcrum of this lever is near the body-tube end of the arm, and within the body tube it moves a nosepiece which carries the objective. As made by the late firm of Powell and Lealand in their famous but costly stands, this form of coarse and fine adjustment has, as a well-known writer said, 'held an unrivalled position for the past fifty years.' But except in the microscopes made formerly by this particular firm, we would strongly urge the reader to avoid absolutely any instrument in which this model is apparently followed, whether it be new or second-hand. As made and exposed for sale in the windows of the average optician, the coarse adjustment is a mean triangular bar with perhaps one edge converted into a rackwork, and showing distinct lateral play even before use, whilst the fine adjustment is a screw at the side of the body tube working on a bracket, and actuating an ill-fitting short tube within. There are still hundreds of such stands for sale in London and the provinces, but they are only sold by and to the ignorant, and a maker of any present-day reputation would be chary of displaying even a second-hand microscope of this type in his window. The coarse adjustment is untrustworthy from the start and rapidly becomes worse, whilst the fine adjustment deteriorates even more quickly. The next form of fine adjustment is that of which the Jackson model may be taken as a type (Fig. 16). Here there is a solid arm projecting over, and firmly bracketed to, the stage. The rackwork of the coarse adjustment is attached to the body tube, and works in V-shaped slides. We have already said that these bearings should be of ample length and properly 'sprung.' Between the slides of the coarse adjustment and the limb or arm is a second set of similar V-shaped

slides forming the movement of the fine adjustment. The body is raised or lowered by means of a lever working against a pin and light spring, and actuated by a fine micrometer screw (Fig. 6), much as in the Powell and Lealand form, except that the whole body instead of an inner tube is moved. The long leverage reduces the weight upon the fine adjustment screw to about one-

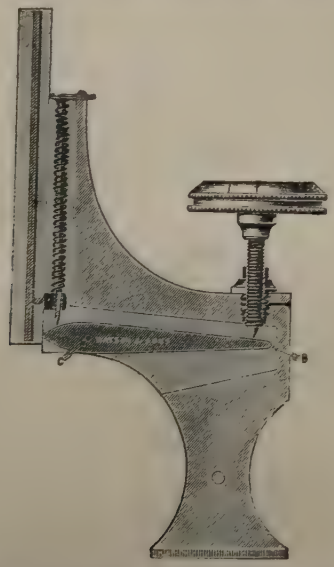


FIG. 6.—WATSON'S HORIZONTAL LEVER FINE ADJUSTMENT.

fifth only of the weight of the body tube and objectives. This is one of the best forms known; it is rigid, extremely sensitive, and not liable to deteriorate rapidly even in unskilled or careless hands. In this form also it is apparent that the distance between objective and eyepiece remains unaltered. This fine adjustment was formerly adopted by Watson and Sons in nearly all their

stands, but is now largely replaced by English makers by a form which provides a milled head at either side, so that it can be actuated by either hand. Watson's form is shown in Fig. 7, and that of other English makers is more or less on a similar principle.

A well-known form of fine adjustment is that represented by the Continental form of direct-acting micrometer screw, which moves the limb as well as the body, the former working generally upon an upright triangular bar. This form is perhaps still too often met with. Its main merit must surely be its cheapness, and we regret that more than one of our leading English makers, who have so often shown the way for Continental makers to follow, have been forced by foreign competition to adopt the cheaper but less efficient design in certain stands. That it is imperfect is proved by the fact that progressive and well-known Continental firms like Zeiss, Leitz, and Reichert, have found it necessary to re-design entirely their fine adjustments in order to bring them up to present-day requirements. Briefly put, the drawbacks of this type of fine adjustment are due to the fact that the whole weight of limb, body tube, objectives, etc., is borne on a single direct-acting screw, which if fine soon wears out, and if coarse is insufficiently sensitive. Moreover, the leverage of the projecting limb accentuates the wear and tear, being assisted therein by the ineradicable tendency of the average student to lift his microscope bodily by this limb.

The imperfections of the ordinary Continental form of direct-acting screw already alluded to are generally guarded against as much as possible by keeping the limb very short, but the increased demands for a larger stand have made the faults of this type of fine adjustment too evident, and therefore all the leading Continental

makers, and those who follow their lead, have now adopted entirely new forms of fine adjustment for their more expensive models, of the ingenuity and efficiency of which we cannot speak too highly, though we think it only just to note that in this they are but following the practice which has been long adopted by the best English makers.

In Zeiss's form of improved fine adjustment, as fitted to their photo-micrographic stand, the milled heads project horizontally on each side of the limb, and bear a worm-screw, which rotates the direct-acting fine

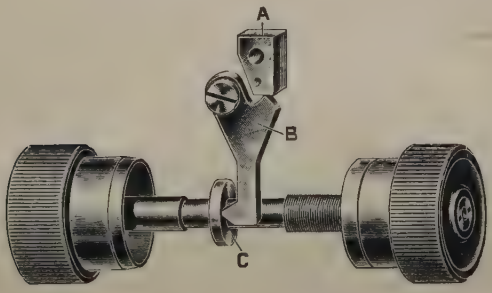


FIG. 7.—WATSON'S VERTICAL LEVER FINE ADJUSTMENT.

adjustment screw by means of a corresponding pinion below the latter, means being adopted to prevent over-running.

In Leitz's form of fine adjustment the milled heads project horizontally on each side of the limb, and bear a worm-screw which rotates a cam running beneath a roller, which carries the weight of the body tube direct. An advantage, or a disadvantage, according to the views of the worker, of this arrangement is that the movement is necessarily continuous, the movement of the tube, either upwards or downwards, being necessarily reversed when the cam has reached the limit of its travel.

Other makers use various forms of micrometer screw and lever, and there are innumerable modifications peculiar to each individual maker, which it is not necessary to recapitulate. The rotation of the fine adjustment should be in the same direction as that of the coarse adjustment.

It will be noted that all these forms of fine adjustment enable the limb of the microscope to be made rigid with the stage, as in the Jackson model, giving much greater firmness and a long length of limb, with corresponding increase in the size of stage. This type of stand is very convenient to lift, whether fitted with a handle or not.

Before leaving the subject, we may mention that when photo-micrography is in view the milled head of the fine adjustment should be grooved, so as to hold a fine cord. It is of advantage to have the edge of the milled head divided, so that, the movement being known, it may be used to gauge thicknesses, to take refractive indices, etc., and it would be of service if the graduations represented the movement of the tube in fractions of a millimetre, instead of merely tenths and hundredths of a revolution of the milled head.

Tube and Tube-Length.—The **Body Tube** is the optical tube which carries the objective and eyepiece. Not the least of the benefits which microscopists owe to the Royal Microscopical Society is the standardizing of the screw of the objective, and the 'Society screw,' slightly modified from that originally drawn up in 1858, is now almost universally accepted by microscope makers throughout the world, necessary standard gauges being formerly supplied at cost price by the Society, though their use is no longer necessary (see Appendix). As a result, 'adapters' to permit of an objective by one maker being used with a microscope by another maker

of possibly different nationality have become things of the past. The size of the tube itself, however, and the diameter of the eyepieces have met with less universal acceptance. The diameter of the tube is variable, but preference should be given to one with a fairly large diameter, not only on account of the draw-tube, but in consideration of the advantage of a fairly wide tube in photo-micrography. Zeiss, for example, fits a specially wide tube to a microscope intended for this purpose alone. The standard length of the English tube is, or rather was, 10 inches (250 millimetres), this length being the normal visual distance of a normal eye. The Continental tube is from 6 to 7 inches (160 to 180 millimetres), and the importance of the difference lies in the fact that an objective is necessarily 'corrected' by its maker for a definite thickness of cover-glass and a definite length of tube. A variation in the thickness of the cover-glass of the object requires a corresponding adjustment in tube-length, according to a practice that will be explained later, unless some means is provided of adjusting the objective itself. The necessity for this correction becomes more marked with high powers. It follows, therefore—and this must carefully be borne in mind—that objectives made or, as it is technically called, 'corrected' for the one tube-length cannot be used for the highest class of critical work with the other tube-length, though low powers with low eyepieces bear the alteration fairly well. It has been stated that an objective corrected for the long tube performs better on the short tube than an objective corrected for the short tube does on the long tube, and in contradistinction to this, that an objective corrected for the short tube bears high eyepiecing better than one corrected for the long tube, and the latter statement

at least is correct. Formerly all English objectives were corrected for the long tube, but now our leading makers have adopted the short tube-length as the standard for their students' series of objectives, owing partly to the increased use of the more compact short-tubed microscope, and to the necessity of conforming to Continental practice born of foreign competition. But many English and some of the best Continental lenses are still corrected for the longer tube-length when required; and to provide for the use of both kinds of objectives, and to give facilities for the means of correction already alluded to, the body tube is made with an inner **draw-tube,** to which a cloth lining, frequently fitted, gives a very pleasant and soft movement. With the exception of certain binocular microscopes, all our more modern English stands have body tubes about 6 or $6\frac{1}{2}$ inches long, generally capable of extension to about 10 inches. Some makers provide a second inner tube, like a telescope, to give an even wider variation, such as from $5\frac{5}{8}$ inches (142 millimetres) to 12 inches (305 millimetres), and the advantage of this is obvious. One of the tubes may even be provided with a rack and pinion for facility in exact adjustment. The Continental and American microscope does not generally extend sufficiently to enable it to be used with objectives corrected for a 10-inch tube, and this is manifestly a drawback, though many English stands of the less expensive type have followed suit. After what we have said, the necessity of a draw-tube is obvious, and it should be graduated in inches or millimetres, to show the amount of extension and for facility in adjustment. We may mention here that the method of measurement of this tube-length is not clearly defined, measurements being variously taken from the upper end of the tube where

the eyepiece is inserted to its lower end containing the screw of the objective (known as the mechanical tube-length), and from the diaphragm of the ocular (which generally varies in position according to the power of the ocular) to the upper focal plane of the objective (known as the optical tube-length), which is about 20 millimetres more. In practice, however, these measurements need not be gone into too closely, as no practical microscopist would adjust his tube-length or objective to make the necessary corrections by any other guide than his estimation of the image under inspection at the moment.

In the older form of binocular microscope the tube is necessarily of a fixed length of about 10 inches, some slight adjustment being provided only for the varying distance between the eyes of different observers; but the newer forms of binocular (see pp. 47 and 86) have now replaced the earlier Wenham type.

The lower end of the draw-tube should be provided with the Society screw, so as to permit of the insertion of objectives having an exceptionally long focus, or of lenses constructed for special purposes, such as the Bertrand lens, or of the lens provided with Zeiss's apertometer.

Diameter of Draw-Tube.—The upper end of the draw-tube contains the eyepiece, which should fit easily, so as to cause no danger of forcing down the tube and objective upon the slide beneath. The diameter of the eyepieces, as already intimated, is not uniform, the Continental size, now adopted in most of our own students' microscopes, being the smallest, whilst the English eyepiece is considerably larger. To endeavour to minimize this state of things, and at the same time to formulate a scheme that would bring all the principal

opticians into line, the Royal Microscopical Society has adopted three (originally four) standard sizes for eyepieces. These are as follows: Small, Large, Extra Large. (For exact sizes, see Appendix.) Of these, the first is the size in general use on the Continent, and has been adopted by English makers for their students' size instruments; the second is the size adopted for the larger English stands, especially binoculars; whilst the third is now but little used. There was formerly a size intermediate between the small and large sizes, but this was only used by one or two makers and is now withdrawn, whilst the extra large size was used by Powell and Lealand. There can be little doubt that the interests of microscopists generally are best served by the adoption of the small and large sizes only, and it is worthy of note, as an evidence of public spirit, that immediately on the original publication of these standards two of our leading makers, Charles Baker and W. Watson and Sons, promptly adopted these two standards, though this necessitated the changing of their gauges, sizes of tubes, etc. There can be no question that the large size, which may be taken as the English size, is preferable to the small or Continental size. This latter is smaller than is necessary, and has consequently certain disadvantages attending it, though it must be admitted that few objectives will bear an eyepiece whose width, when uncurtailed by a diaphragm, utilizes the extreme peripheral rays of the objective.

CHAPTER III

THE CHOICE OF A MICROSCOPE

HAVING endeavoured to explain as fully as possible the principles of design in the modern microscope stand, it will be advisable to give a practical demonstration of our remarks by briefly passing in review some of the stands of various leading makers, with a view to assisting in the choice of an instrument. It is, of course, impossible to mention every microscope upon the market, and any particular instrument is selected as a type rather than as necessarily superior to others, but it is a noticeable fact that those instruments which are especially distinguished by excellence of design and workmanship are made by a comparatively small group of makers, perhaps four in England, as many on the Continent, and two or three in America. These makers are constantly striving to perfect their instruments, both mechanically and in their optical parts, and deserve special notice accordingly. We are therefore doing no injustice to others, as matters stand at present, in advising the prospective purchaser of a microscope to confine his choice to well-recognized makers, *to buy no instrument that does not bear a name upon it,* and in particular to be cautious in deciding to buy any instrument without a most careful consideration of its real value. There are many microscopes offered for sale by firms of repute in other branches of optical and scientific manufacture that are quite unworthy of the name of microscope, as the term is understood by serious workers with this instrument, and of which but a little

experience is needed to show their inferiority to the best instruments of their class, types of which we are about to deal with specifically. This unfortunately applies to many microscopes offered in both small and large shops and stores, which no serious worker would care to possess. The reader who has carefully perused the preceding chapter will be in a position to understand the terms used in describing a microscope, and will appreciate for himself the advantages of the respective instruments; but as a microscope is by no means an inexpensive instrument, and the purse may not necessarily be a deep one, it may be as well if we give here the table of relative importance of the various parts which appears in Carpenter's 'The Microscope and its Revelations,' as drawn up by the late Dr. Dallinger.

Relative Importance of Microscope Parts.

1. A coarse adjustment by rack and pinion.
2. A sub-stage.
3. A fine adjustment.
4. Mechanical movements to sub-stage—*i.e.*, focussing and centring.
5. Mechanical stage.
6. Rackwork to draw-tube.
7. Finder to stage.
8. Plain rotary stage.
9. Graduation and rackwork to rotary stage.
10. Fine adjustment to sub-stage.
11. Rotary sub-stage.
12. Centring to rotary stage.

Few workers will wish to alter materially the order in which these stand, unless, perhaps, to put No. 6 rather lower down in the list, or to insert the provision of a sliding-bar to the stage as an alternative before No. 5. The order of Nos. 9 and 12 might also possibly be reversed.

Simplest Form of Microscope.—Our simplest microscope is, then, a microscope with a plain stage, mirror, and coarse adjustment by rack and pinion, but without fine adjustment, sub-stage attachment for condenser, or means of inclination, so that it must always be used

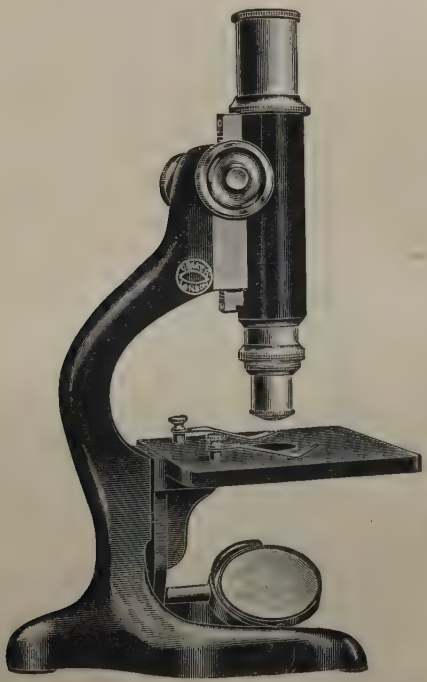


FIG. 8.—BAKER'S 'SCIENCE' MICROSCOPE.

in the vertical position. Such a microscope is made by Baker (Fig. 8), Bausch and Lomb, and the Spencer Lens Company. As a laboratory microscope for rough work such an instrument is a most useful possession, and it is capable of good work of other kinds up

to a certain degree, for there is no question that a good and well-made coarse adjustment is a more satisfactory matter than an ill-designed fine adjustment.

The next type is naturally a microscope similar to the foregoing, but with a fine adjustment and a sliding-tube

FIG. 9.—BAKER'S 'STUDENTS'' MICROSCOPE.

coarse adjustment. They are still too frequently met with in our medical schools and elementary science laboratories, and the best that can be said in their favour is that they are cheap, and that the perfunctory way in which the use of the microscope as a microscope is taught

in such laboratories renders it inadvisable to hand over anything better to the tender mercies of those who use them. We have observed that under such circumstances the fine adjustment is nearly always used to focus the low as well as the high powers, and it is small wonder that the screw of the fine adjustment soon shows marked evidence of such use. This may be an argument against providing better instruments, but it seems to us rather an argument for providing more information as to the use of the microscope, which is still too often looked upon as a mere magnifying-glass. When a greatly superior instrument can be obtained for a very small extra cost, it is bad policy to purchase the cheaper instrument.

Students' Microscopes.—In inclinable microscopes fitted with both coarse and fine adjustments, together with a sub-stage, we have a large and indeed a bewildering choice, all distinguished (in the makers' catalogues, at least) by arbitrary names, letters, or numbers, a mere variation in the type of mechanical stage being considered enough to justify a new label. Dealing with the less expensive stands first, and taking the leading English makers in alphabetical order, we have Baker's 'Students'' microscope (Fig. 9), fitted with coarse adjustment by rack and pinion, fine adjustment of the side type, with rigid limb, draw-tube extending to 7 inches, and horseshoe foot. The stage is large, and beneath it is fitted a ring to hold a condenser and iris diaphragm. For more advanced work we have the 'D.P.H.' stand (Fig. 10), provided with Continental size eyepieces. The limb is rigid, and has a handle for facility in carrying; the fine adjustment works at the side, and actuates a lever, by means of which a very smooth and sensitive movement is given.

The foot is of the 'claw' type, which is very nearly as steady as the tripod, and gives more room beneath the stage. The instrument is supplied with plain sub-stage tube for condenser, screw focussing sub-stage, or

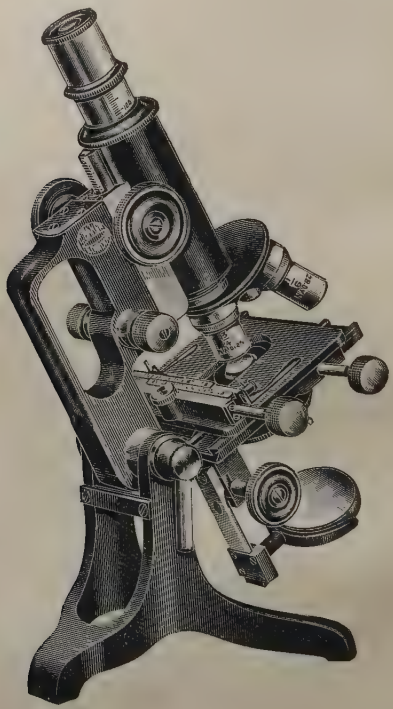


Fig. 10.—Baker's 'D.P.H.' Microscope.

rackwork focussing and centring sub-stage, as required, and can be had with a removable or built-in mechanical stage at choice. This is an excellent microscope, combining many of the best features of both English and Continental models, but centring screws should be

supplied to the sub-stage as a standard instead of as an extra.

A rather more elaborate microscope is the 'R.M.S.,' which can be provided with rackwork to the draw-tubes, and has a rotating stage. The body here is fitted with

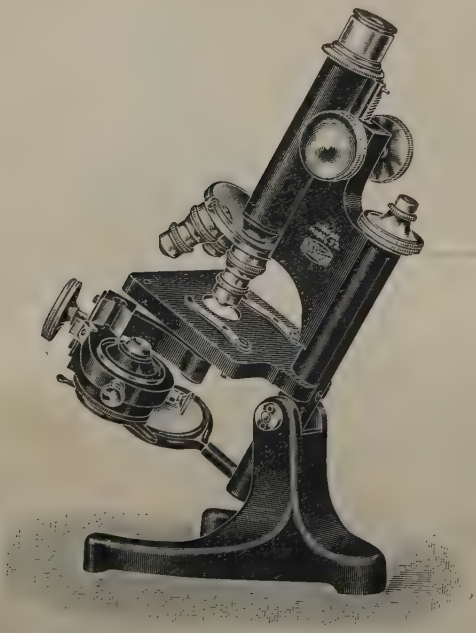


FIG. 11.—BECK'S SMALLER 'LONDON' MICROSCOPE.

a plain slide, which takes an interchangeable binocular body, and is also useful when very low-power objectives with long focus are being used.

All these microscopes are of excellent workmanship and design, and the last two are suitable for almost any kind of work.

R. and J. Beck's best-known microscope of this class is their 'London' stand. The design of the cheaper models is of the Continental type, as the makers recognize the hold this kind of microscope has in our medical schools and university laboratories. The criticisms levelled at the horseshoe foot and at the

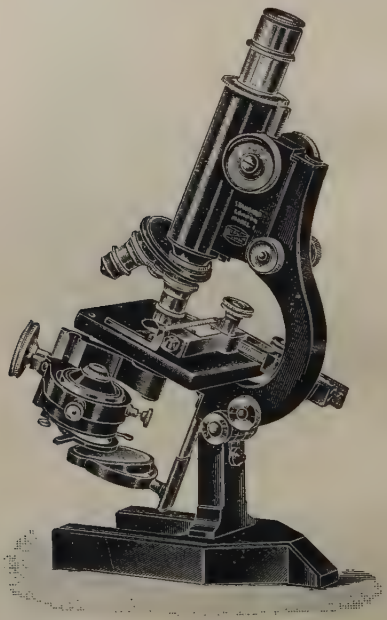


FIG. 12.—BECK'S 'STANDARD LONDON' MICROSCOPE.

triangular bar and micrometer screw fine adjustments, carrying both limb and body tube, apply, therefore, to this model, but our criticism need go no further than this. The workmanship is good, and the price extraordinarily low, whilst by bringing the pillar forward and placing the inclining joint immediately beneath the

stage greater stability is given to the whole stand in various positions. This microscope is made in various degrees of completeness, from a plain stand, with coarse and fine adjustment and swing-out sub-stage, to a larger model, with removable mechanical stage and

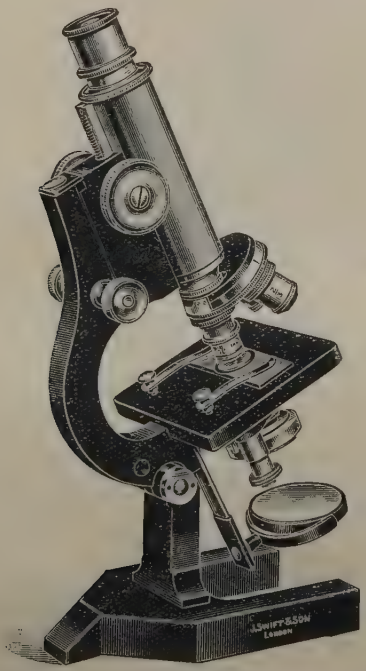


FIG. 13.—SWIFT'S 'B.S.G. STANDARD' MICROSCOPE.

compound sub-stage. Fig. 11 illustrates the smaller model.

A larger model of the 'London' microscope, known as the 'Standard London,' is fitted with the more modern type of limb and side fine adjustment. It can

be obtained with a simple stage only, or with a removable mechanical stage and compound swing-out focussing and centring sub-stage, as illustrated (Fig. 12). It can also be supplied as a binocular microscope of

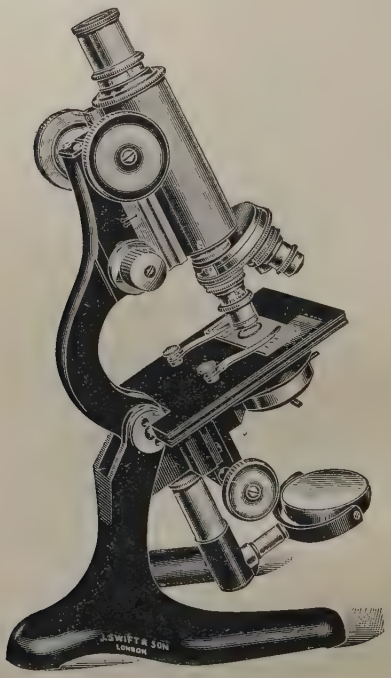


FIG. 14.—SWIFT'S 'ARMY' MICROSCOPE.

modern type, specially designed and constructed by this firm.

J. Swift and Son make several types of microscopes, suitable for histology, physiology, bacteriology, etc., of which the 'B.S.G. Standard' (Fig. 13) may be taken as a type. It has the horseshoe type of foot, the coarse

adjustment is by spiral rack and pinion, and the fine adjustment is by micrometer screw and lever. The stage is covered with vulcanite and provided with clips. A non-centring ring beneath the stage carries the condenser, and a spiral screw adjustment is supplied, which also serves to swing the condenser out of the field of view if required. The body is of large diameter for photo-micrography with low powers, and the draw-tube is graduated, and extends from 160 to 200 millimetres.

Messrs. Swift also make a somewhat similar microscope known as the 'Army' (Fig. 14). It has a claw stand, and a large stage measuring 95 by 105 millimetres, and this has finder lines engraved on it, and can be fitted with a very handy sliding-bar running on rollers in parallel grooves at the sides, or with attachable mechanical stages. Both these microscopes can be fitted with centring screws and a fine focussing adjustment to the sub-stage, the latter ingeniously working on the same spindle as the coarse adjustment.

Swifts make also larger and more elaborate stands, including some of unusual design, the merits of which perhaps scarcely compensate for the increased cost, though the 'Wales' principle of mounting the body and stage at opposite ends of a semicircle, which itself is held by the foot in a clamp in any required position, is worthy of notice.

The microscopes of these makers are of excellent workmanship in every way, and they have been pioneers in many improvements in microscope design and detail.

W. Watson and Sons have several types of instruments which come under the description of students' microscopes. The cheapest of these is known as the 'Kima,' but a rather better type is the 'Praxis' (Fig. 15). In outward appearance it looks like a microscope of the

Continental type, again perhaps a concession to the prevailing ignorance of other than Continental types in many of our medical schools; but the limb is really cast solid with the stage, and the milled head of the fine adjustment actuates a lever of Messrs. Watson and

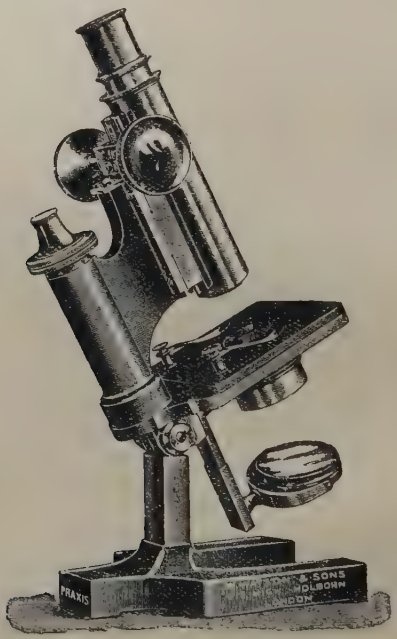


FIG. 15.—WATSON'S 'PRAXIS' MICROSCOPE.

Son's well-known sensitive and reliable pattern, which is much superior to the ordinary Continental direct-acting screw (see p. 19). There is the usual coarse adjustment by rack and pinion; all these fittings are sprung, and have adjustments for wear and tear. The stand is of the Continental horseshoe type, but is cast

solid with the pillar, and is blackened in a way which makes it impervious to acids, etc. The draw-tube extends from $5\frac{3}{4}$ to 9 inches. The stage is covered with vulcanite, and there is a sub-stage ring for the condenser, which enables it to be swung aside, or a spiral screw focussing

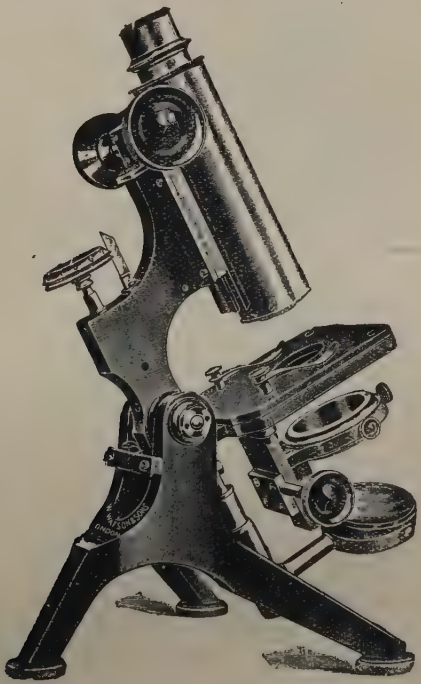


FIG. 16.—WATSON'S 'EDINBURGH, F' MICROSCOPE.

sub-stage, or even a compound sub-stage, can be fitted if required. This is an excellent instrument for laboratory work, and calculated to stand the maximum amount of rough or unintelligent usage.

A somewhat similar but larger form of microscope

of this kind, with side fine adjustment, is known as the 'Service,' and to this most of the refinements already enumerated can be provided.

This firm's best-known stand is, however, without doubt their 'Edinburgh Student's' microscope, which

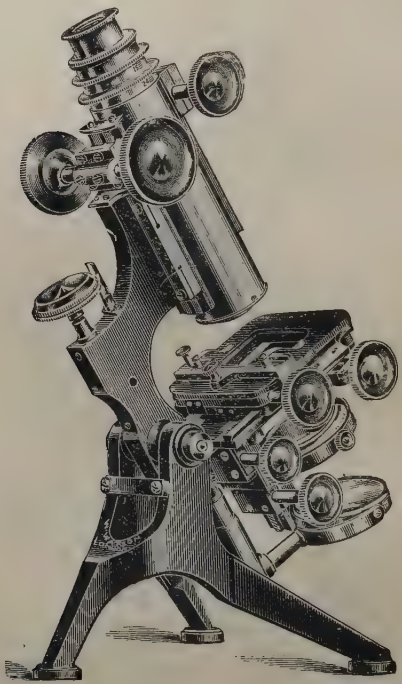


FIG. 17.—WATSON'S 'EDINBURGH, H' MICROSCOPE.

for excellence of design and workmanship, combined with moderate cost, fully deserves the wide recognition it has gained. It is made with both horseshoe and tripod foot, as illustrated in Fig. 16. This is the 'F' model, the graceful appearance of which is at once

apparent. The fine adjustment is of the now well-known lever form, which is sensitive to the $\frac{1}{30000}$ of an inch. The milled head is graduated. The draw-tube is also graduated, and extends from $6\frac{1}{2}$ to 10 inches, the body being $1\frac{1}{2}$ inches in diameter. The stage is $3\frac{1}{2}$ inches square. There is a compound rackwork (focussing and centring) sub-stage.

Not the least of the advantages of this microscope is the facility with which various further refinements can be added without overburdening it or raising the price to a prohibitive figure. These refinements include a mechanical stage built as part of the microscope, and therefore liable to a minimum of wear and tear, a sliding-bar to the stage with movable stop, graduations to the mechanical stage with verniers to read up to $\frac{1}{10}$ millimetre, and a fine adjustment to the sub-stage (Fig. 17).

Somewhat similar instruments fitted with side fine adjustment are known as the 'Research' and 'Bactil.'

The examples given will show the variety of design in the students' class of microscope, and that in their most elaborate form they approach closely to the very highest type.

Research Microscopes.—But those who desire and can afford to pay for the very best microscope that can be obtained will find it in the more expensive instruments made by the makers already enumerated, of which instruments Watson's 'Van Heurck' (Fig. 18) may be given as an example. But the tendency of the day is to use a more compact type of microscope without undue multiplication of refinements. Amongst these instruments of the finest type one cannot refrain from mentioning, with regret that it is no longer made, the famous stand made by the late firm of Powell and Lealand, which for many years held an acknowledged

position amongst microscopes of the very highest class, both for design and for workmanship, and the only criticisms that can be made are against its weight, and that its large size renders it inconvenient to use at an ordinary table, except in the inclined position. It is noteworthy that this stand for years remained almost unaltered, whilst all other instruments were undergoing transition, a striking proof of the excellence of the design

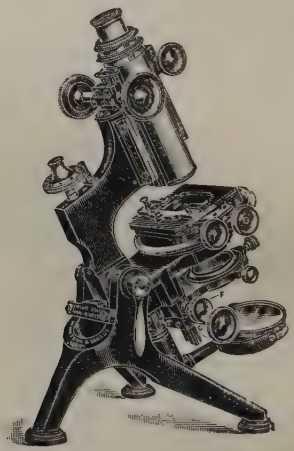


FIG. 18.—WATSON'S 'VAN HEURCK' MICROSCOPE.

and of the sound judgment, so much in advance of the times, of the designers.

Continental Microscopes.—Our notices of modern microscopes have been limited to a few of the leading makers, for reasons already stated; but our review would be incomplete if we confined ourselves entirely to English stands, if only on account of the large sale in this country of certain Continental microscopes. The causes of the popularity of such microscopes in our

medical laboratories in particular are not quite easy to state. We should be inclined to suggest that, whilst there can be no doubt that the majority of improvements in microscope stands have unquestionably emanated and still emanate from England, our makers have in the past confined themselves too much to making costly stands, fitted with elaborate refinements, and requiring considerable knowledge for their utilization, and have devoted but little attention to the requirements of the ordinary worker or student, who uses his microscope with but little appreciation of its higher possibilities, and to whom the consideration of cost is a very serious item. The result has been a large demand for an inexpensive but well-made stand, provided very frequently with neither condenser, coarse adjustment, nor means of inclination, but calculated to stand rough wear and tear. That the best-known Continental makers are more and more following an English lead in the improvements they are adopting to their microscopes is perhaps evidence not only of a fuller realization of the superiority of the English model, but implies also that our manufacturers in turn are now fully alive to the state of affairs, and recovering lost ground.

Since the first edition of this book was published the Continental microscope has come still more into line with English practice, and the more expensive microscopes of the leading Continental makers show great improvement in design, combined with an excellence of workmanship and an attractiveness in finish, which leave little to be desired. A careful comparison of price-lists will show, however, that, contrary to the prevalent opinion, an English microscope of good quality can be obtained for a less rather than a greater sum as compared with a Continental instrument. We believe, therefore,

that better value can be obtained by buying an English microscope, both in excellence of design and in price, but in saying this we have no wish to disparage the present-day Continental microscope, and with regard to objectives it will be seen later that we can claim no superiority over Continental objectives, and that we owe much of the improvement in our own objectives to new forms of glass introduced at Jena (see pp. 73 and 134).

It will perhaps be of service if we give here the distinctive features of the Continental as compared with the English stand.

The foot is of the horseshoe type, the instability of which is in certain cases somewhat reduced by bringing the stand farther forward beneath the stage, and the fine adjustment was formerly of the direct-acting micrometer screw type, moving the whole limb upon a triangular bar, the disadvantages of which we have already pointed out. The Continental makers have now, following the English lead, shown their recognition of these disadvantages by designing improved forms of fine adjustment (see pp. 17-22), and these are perfectly satisfactory. The eyepieces are in all cases of the Continental size—*i.e.*, 0·9173 inch—but the draw-tube is generally of insufficient extension to enable other objectives to be used than those corrected for the short Continental tube. In the larger stands a most elaborate form of sub-stage is provided. It is focussed by rack and pinion, but the focussing adjustment sometimes carries also the tail-rod of the mirrors, and moves them at the same time—a manifest disadvantage—whilst the mirror itself is frequently non-adjustable on the tail-rod. A small iris diaphragm is provided in certain stands *above* the condenser and immediately beneath the stage, which is to be used only when the condenser

is removed, and to make the exchange the more readily the condenser itself is mounted on a hinge at one side, so that it can be lowered from the fitting and swung out of the way and upside down. Beneath the condenser when in use is a larger iris diaphragm, which can not only be swung completely aside, but is capable of adjustment horizontally by means of a special rack and pinion, with a view to giving oblique illumination when requisite (Fig. 21). This arrangement, though comparatively a recent development, has been adopted by most makers of the Continental form of instrument, but it is, in our opinion, an unnecessary complication (see Chapter V., p. 97). With all this elaborate means of giving *oblique* light, no means of centring the condenser (a matter of incomparably greater importance) is generally provided, and when specially ordered is usually cumbrous. An achromatic condenser is seldom supplied; in fact, the Continental maker apparently fails to realize the advantages of critical illumination, which is so necessary if the beautiful corrections of his own fine objectives are to be realized to the full (see p. 90).

The best-known Continental makers are Ernst Leitz of Wetzlar, Carl Reichert of Vienna, and Carl Zeiss of Jena; whilst the Bausch and Lomb Optical Co. and the Spencer Lens Co. are the most prominent firms in America. R. Fuess of Steglitz, Voigtländer of Brunswick, Otto Himmler and Hartnack of Berlin, R. Winkel of Göttingen, F. Koristka of Milan, and Nachet Fils of Paris, are less known here.

We shall content ourselves, therefore, with enumerating one or two microscopes by Leitz and Zeiss on the Continent, and by Bausch and Lomb, and the Spencer Lens Co. in America, as types of Continental and American stands.

Leitz's cheaper microscopes are of the usual Continental type, with direct-acting micrometer screw fine adjustment actuating both limb and body, and with spiral screw focussing sub-stage. The foot is of the horseshoe

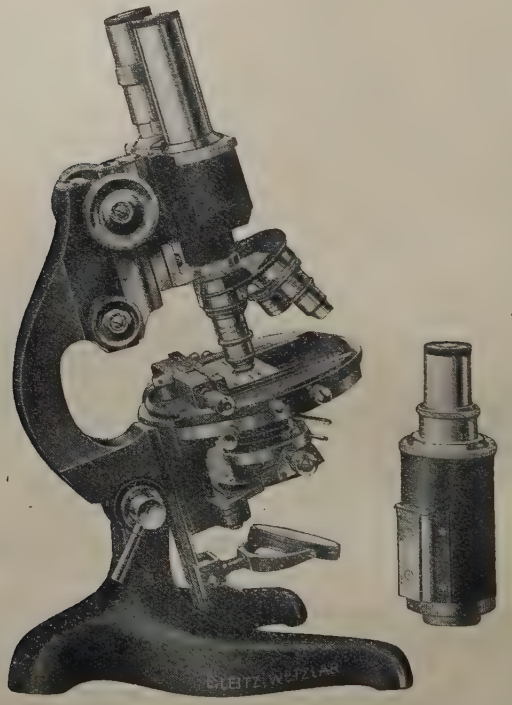


FIG. 19.—LEITZ'S 'A.A.B.M.' MICROSCOPE.

or similar type. Somewhat larger but similar stands can be obtained with rack and pinion focussing sub-stage, but without centring screws, with the decentring iris diaphragm already alluded to, and with the horseshoe foot or with the English claw foot. The tail-rod of the

nirror is, unfortunately, carried by the sub-stage, and noves with it. The newer stands, however, contain citz's new form of fine adjustment, and a rigid limb iving a large stage space. Stand 'A,A.B.M.' (Fig. 19) is fair example of this kind of stand, embodying many of he particulars already detailed, with a mechanical stage,

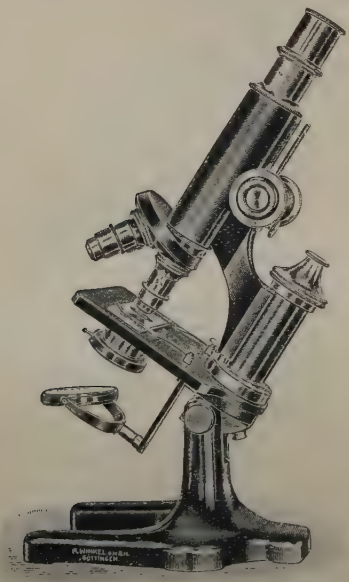


FIG. 20.—WINKEL-ZEISS 'W.B.C.' MICROSCOPE.

nd moreover illustrates a type of microscope which is ecoming increasingly popular, as it has interchangeable onocular and binocular bodies. More elaborate stands y the same maker contain most modern refinements, cluding centring screws to a rotating stage, but there still a curious disinclination to fit centring screws to

the condenser, probably accounted for by the fact that the German still clings to the uncorrected Abbe illuminator, with all its aberrations. In the larger stands the condenser is adjustable by rack and pinion, and has the lateral adjustment to the iris just mentioned, and this swings aside to allow the condenser in turn to swing

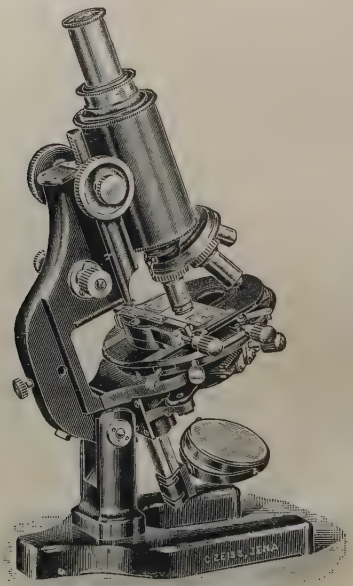


FIG. 21.—ZEISS'S 'C.C.E.' MICROSCOPE.

out from beneath the stage. The workmanship of all these stands is excellent.

Reichert's microscopes are very similar in appearance to Leitz's new stands.

Zeiss's smaller stands are of the usual well-known Continental type (*cf.* Fig. 20), but the larger and newer

models are fitted with Zeiss's new fine adjustment and rigid limb. Stand 'C.C.E.' may be taken as a type (Fig. 21), the illustration of which explains itself. The

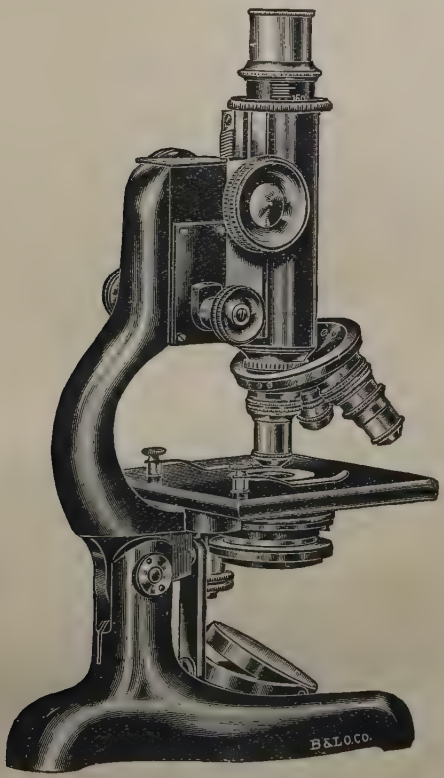


FIG. 22.—BAUSCH AND LOMB'S 'F.F.S.' MICROSCOPE.

body is wide for photo-micrography, there is an efficient mechanical stage (Fig. 21), the sub-stage is adjustable by rack and pinion, and the tail-rod of the mirror is fixed; but there are no centring screws for the condenser

(though these can, if specially ordered, be fixed in a rather cumbrous way to the condenser itself), and the iris diaphragm can be decentred laterally, as already described, and also swung aside, together with the condenser. The foot is of the horseshoe type, and the instrument is therefore not very steady in the horizontal position, and needs clamping down. The workmanship and finish is of the highest class.

American Microscopes.—The Bausch and Lomb Optical Co. and the Spencer Lens Co. of America make models following very closely the Continental type and of excellent workmanship, whilst the finish is particularly attractive. Certain models have solid arm, with handle for lifting, and lever fine adjustment of the Jackson type. Messrs. Bausch and Lomb's 'F.F.S.' stand, illustrated in Fig. 22, may perhaps serve as an example of their students' stand. The large stage and swing-out adjustment to the condenser will be noted. This microscope can be supplied with simple sub-stage ring and iris diaphragm beneath the stage, or with rotating stage with centring screws and removable mechanical stage, but none of these models appear to be fitted with compound rack and pinion focussing and centring sub-stage. Similar stands are made by the Spencer Lens Co., and of the more elaborate stands made by both these firms the Spencer Lens Co.'s 'No. 15' microscope (Fig. 23) is illustrated as a type, and with what we have already said the illustration is self-explanatory. But these makers have designed a compound sub-stage which provides not only rack and pinion and centring screws, but also a fine adjustment, and instead of having a ring for the condenser a fork open in front is fitted, so that the condenser is easily slipped in and out, being held in place by two side springs, or a polarizer or other optical apparatus can be

inserted. In addition there is a stage iris diaphragm, and an adjustable iris diaphragm which closes on one side only to give any desired oblique illumination.

Choice of a Microscope.—From the types of microscopes just described, and which conform to present-day requirements, the reader can be left to make his own

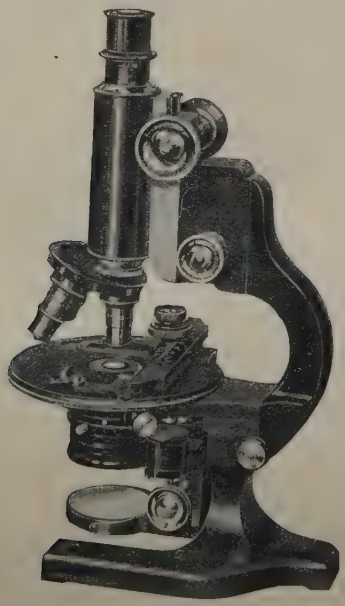


FIG. 23.—SPENCER LENS CO.'S 'NO. 15' MICROSCOPE.

selection, or from their careful study he will have learnt what to look for in stands by other makers. The list may seem to be too numerous, but it is fairly representative, and the particulars given, when read in conjunction with the preceding chapter, should help the worker to make a wise and suitable choice. We have been asked

frequently, Which is the *best* microscope ? but to this question there is no justifiable answer. One might as well ask which is the best gun or motor-car. The purchaser will be guided by his predilections, his requirements, and by the depth of his purse. Of course, money at first cost may be saved by buying, for instance, a microscope without a condenser, as in the pretentious stands one meets with in so many opticians' windows, but it is to save the beginner from the mistake of making such a purchase that this part of the present book has been mainly written. We have before said, and it cannot be too often repeated, that a condenser is absolutely necessary if the fine objectives of the present day are to be taken full advantage of, and high-power objectives, such as $\frac{1}{12}$ or $\frac{1}{8}$ inch, could scarcely be used alone. Sooner or later, therefore, the condenser fittings become a necessity. The fine adjustment is of equal importance, whilst the provision of a mechanical stage (so often asked for) is really of secondary importance, though undoubtedly of great service to medical and other workers.

The Binocular Microscope.—Keeping in view the acquirement of a stand which can be used for serious practical work, we have dealt only with the monocular, and not the binocular, stand as formerly met with, which, convenient as it is in many ways, and beautiful as are the images which it gives with low powers, has drawbacks which make it unsuitable for general use. These drawbacks are mainly that it has necessarily a fixed tube-length of 10 inches, adjustable only within narrow limits for variation of the width between the eyes of different observers, and that it can only be used as a binocular with objectives up to about $\frac{1}{2}$-inch focus, or, say, 40 degrees air-angle (N.A. 0·34), without great loss

of light. The Wenham type of prism is generally arranged for withdrawal, in which case the microscope becomes a monocular, but with practically fixed tube-length. In consequence, the old binocular is now little met with outside this country, though it certainly has its uses. With low powers the images it gives are strikingly beautiful, owing to their apparent stereoscopic effect, and this latter gives a peculiar depth of vision that is not infrequently of real service to the microscopist by enabling him to realize the contours of minute objects, especially opaque ones, and to grasp the relations of parts in transparent objects in a way that the most careful focussing with the monocular can do only with difficulty. We will content ourselves, therefore, with mentioning that nearly all the English stands described can be obtained with such a binocular body if so required, and that for those who can afford it the addition of a spare binocular body or binocular eyepiece, to replace the monocular and to work in the same fittings, might be an advantageous addition to the microscopical outfit. One or two other forms of binocular microscope will be mentioned in dealing with microscopes for special purposes, without which our review would be incomplete.

But more recently entirely new types of binocular microscopes, or rather binocular eyepieces, have been constructed for use with high powers, and these are very efficient and restful to use, and have become very popular. They are described more fully on p. 86, where their advantages and disadvantages are also dealt with. But in the meantime we may say that most of the more elaborate stands, on the Continent and in America, at any rate, are now sold fitted with this new type of binocular as well as monocular body (see Fig. 19).

Portable Microscopes.—Of such special microscopes, first, in point of importance, come folding or travelling microscopes. Until quite recently these were for the most part defective in design.

Charles Baker makes an excellent travelling microscope called the 'Diagnostic.' It was originally designed for Major Ronald Ross for the study of malarial fever parasites (Fig. 24). It stands on a tripod with a spread of 7 inches, is fitted with rack and pinion coarse and

FIG. 24.—BAKER'S 'DIAGNOSTIC' FOLDING MICROSCOPE.

side screw fine adjustment, whilst the main tube works also in a sleeve to give further latitude for adjustment. There is a draw-tube extending to 170 millimetres ($6\frac{3}{4}$ inches), taking eyepieces of 'R.M.S. No. 1' gauge, square stage $3\frac{3}{4} \times 3\frac{1}{4}$ inches, with clips, rack and pinion focussing and centring sub-stage with condenser, and the usual plane and concave mirrors. The whole packs into a neat leather case, with straps, which measures only $9 \times 4\frac{1}{2} \times 4$ inches. A specially designed removable mechanical stage can be added. The writer has used

one of the early types of this instrument for many years when a travelling microscope was required.

Similar microscopes, but with less elaboration, are made by Watson and by Swift.

The portable microscope of most Continental and American makers is merely a standard stand in which the fork of the horseshoe stand folds together, so that it occupies little less space than the ordinary microscope, but that made by the Spencer Lens Co. (Fig. 25), whilst still a standard model, fitted with full-size stage, focussing sub-stage, and standard limb with coarse and fine adjust-

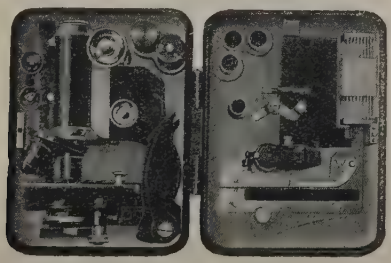


FIG. 25.—SPENCER LENS CO.'S PORTABLE MICROSCOPE, No. 60.

ment, folds into a case $8\frac{3}{4} \times 6\frac{1}{2} \times 3\frac{3}{4}$ inches, and yet has room for a hæmoglobinometer and a hæmocytometer, or special camera lucida.

Leitz makes a large and small travelling microscope, both of the Continental type. The foot folds in a V shape and the mirror and stage are removable.

Petrological and Chemical Microscopes.—These are made now by nearly all the leading makers, and it may be of service if a description is given of a petrological and chemical microscope constructed for the author by Watson, which was fully described in an article

contributed to the *Illustrated Annual of Microscopy* for 1900 (Fig. 26). The description gives the essentials of the microscopes made for petrological purposes, though this microscope, being used mainly for micro-chemical analysis, was provided with a glass stage carefully

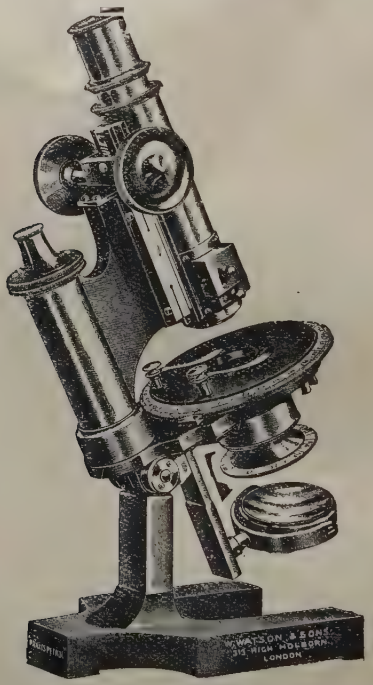


FIG. 26.—WATSON'S PETROLOGICAL MICROSCOPE.

extended to cover the graduations. The objectives used for such purpose are of the less expensive students' type, and when working with reagents likely to give off injurious gases the front lens can be protected by the simple device of attaching a small piece of thin cover-

glass by glycerine or balsam. A means of exactly centring the stage with the optic axis of the microscope is provided, as the centres of various objectives always vary more or less, and this may be either by centring screws to the stage or by a centring nosepiece for the objectives. Beneath the stage is a fitting for condenser and polarizing apparatus so arranged as readily to swing out. The polarizer is graduated on its circumference and provided with stops at each 90 degrees of rotation, and the optical part of the condenser is so arranged as to fit above the polarizer when it is necessary to increase the light for high or medium powers. Above the polarizer can also be placed a removable high-angle condenser system for examination of crystals, etc., in convergent light. The analyser is placed above the eyepiece (not shown in the illustration), both for convenience and to increase the light, and it also is accurately divided on its circumference. Both it and the polarizer are pinned so as to insure the uniformity of their positions. In ordinary microscopes the analyser is often fitted into a ring which screws above the objective. The eyepiece is provided with cross-wires, and the two Nicol prisms are accurately placed so that their principal sections coincide with these cross-wires. Between the eyepiece and the analyser can further be placed a calc-spar plate for stauroscopic examinations. The draw-tube draws right out, and is provided with a Bertrand lens for use with the eyepiece, in order to magnify the interference figures shown by convergent polarized light. In a slot in the body slides a Klein's quartz plate for distinguishing weakly refractive crystals, and a quartz wedge and quarter undulation plate for determining the positive or negative character of crystals. A bi-quartz eyepiece is sometimes added. Finally, the pitch

of the fine adjustment is definitely known and the milled head graduated, as by using a high power the refractive index of thick specimens may by this means be approximately determined. Accessories for taking micrometric measurements and a simple form of camera lucida are desirable additions.

The firms of Beck, Swift, and Watson have paid particular attention to the design and manufacture of petrological microscopes, and their catalogues contain instruments much more costly than the microscope just described, and fitted with many additional refinements. Space will not permit of a detailed description, but we must mention in passing the elaborate instruments made by Swift, including two models embodying an ingenious arrangement of Mr. Allan Dick, in which the stage remains stationary whilst the body tube and sub-stage rotate together. For further particulars the reader is referred to the catalogues of the various makers.

Metallurgical Microscopes.—The great development of recent years in the microscopical examination of metals has led to the introduction of many forms of microscope specially designed for this purpose. As in nearly every case the surface of the metal is to be examined, it follows that a totally different type of microscope is required. For this reason, means of transmitting light through the object are less necessary, whilst the surface to be examined needs strong illumination from above. With low powers this is easy, but with immersion lenses an ingenious arrangement is utilized for transmitting light from above and through the objective, by means of an aperture in the side of the tube containing a reflector (see p. 104). The adjustment of light once made must therefore not be lightly disturbed, and to

obviate this the stage is generally provided with focussing mechanism. Suitable stands are made by Baker, Beck, Swift, Watson, Bausch and Lomb, the Spencer Lens Co., and Zeiss, but the accompanying illustration (Fig. 27), showing a simple microscope by Beck, will

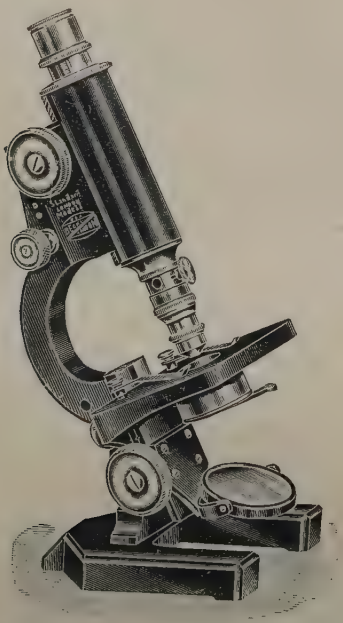


FIG. 27.—BECK'S METALLURGICAL MICROSCOPE.

serve as an illustration. The aperture above the objective will be noted. The stage itself has rack and pinion focussing adjustment. Swift makes a microscope which can be fastened direct to a forging, etc., in the lathe, whilst more elaborate instruments are made by all the firms mentioned, and other instruments are specially

designed for use and photography in the horizontal position. Swift also makes a neat little microscope,

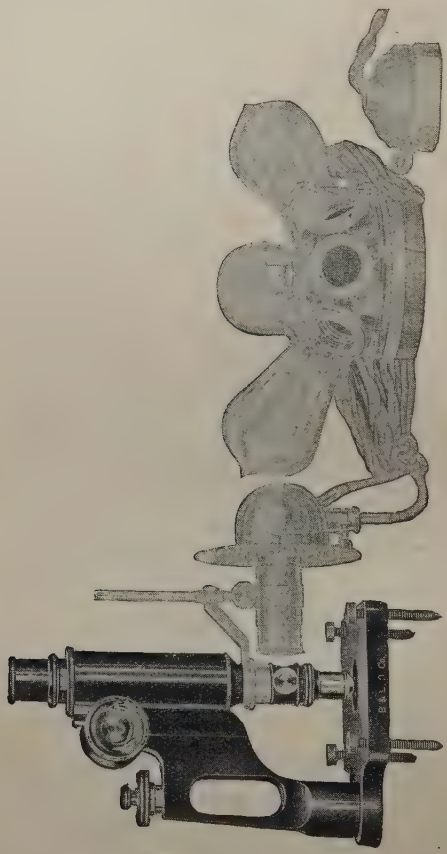


FIG. 28.—BAUSCH AND LOMB'S METALLURGICAL MICROSCOPE.

designed by Mr. J. E. Stead, for use in the workshop, combined with a silver reflector and electric lamp, and a small portable dry battery, but perhaps the neatest and

best-finished microscope of this type is that made by Bausch and Lomb (Fig. 28).

Dissecting Microscopes.—Amongst microscopes designed for special purposes must be included dissecting microscopes, which range from a simple hand-lens, mounted on a stand, to elaborate compound instruments. Of these, the most noteworthy is the binocular microscope designed by Mr. Stephenson, and made by J. Swift and Son. The illustration (Fig. 29) will show

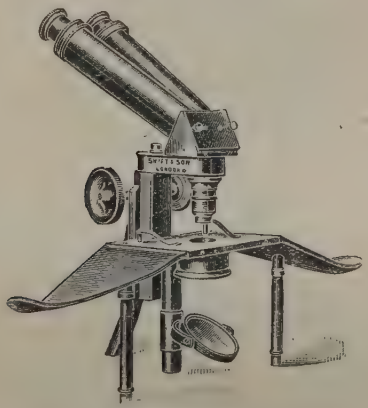


FIG. 29.—SWIFT'S 'STEPHENSON' BINOCULAR DISSECTING MICROSCOPE.

this instrument better than a description. There are two prisms above the objective, so that the light is equally divided in each tube, whilst the image is reflected into the tubes by means of a mirror and so *erected*. The inclination of the tubes obviates the necessity for inclination of the body, and a fine adjustment is not necessary, as high powers are not used with this type of binocular. There is a sliding adjustment for width between the eyes, and a plain sub-stage ring for con-

denser, etc. Being specially designed as a dissecting microscope, it is furnished with leather-covered hand-rests. It forms the most convenient and efficient instrument of its class known to the author, and is unsur-

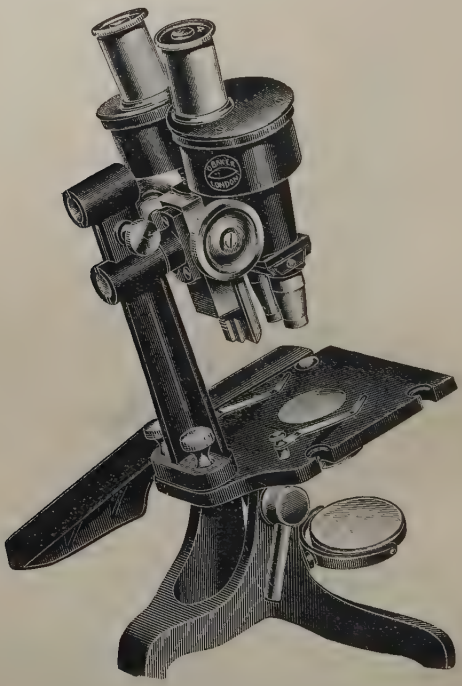


FIG. 30.—'GREENOUGH'S' BINOCULAR DISSECTING MICROSCOPE.

passed for delicate dissections. As a low-power binocular microscope it seems to us to fulfil every requirement if provided with proper condenser.

Greenough's binocular (Fig. 30) is a simple upright stand without sub-stage, and carrying a separate objective at the bottom of each tube. The tubes are set

at an angle to each other so as to bring an independent image of the object into focus in each tube, and it is thus really a combination of two microscopes. It has rack and pinion focussing adjustment. The objectives are from about $2\frac{3}{4}$ to 1 inch focus, and the instrument has the manifest drawback of being confined to these magnifications, and requiring different pairs of lenses for each magnification, though a pair of high-power eyepieces can

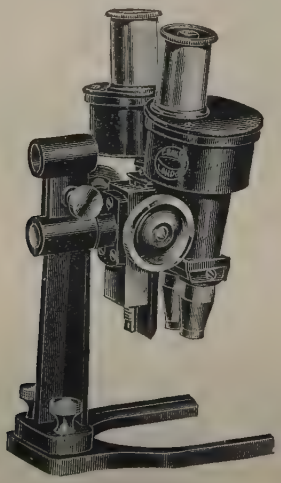


FIG. 31.

vary them from 8 to 72 diameters. It is, however, a most efficient instrument, and gives true stereoscopic vision. As made by Baker, Watson, Leitz, and other makers, the instrument has rackwork adjustment for focussing, and a pair of Porro prisms, of similar design to those in use in the now familiar prismatic binocular field-glasses, are used to erect the image, and by their rotation afford a ready means of adjusting the inter-ocular distance. By the removal of two thumbscrews the limb

can be removed from the stage and stand and fitted to a plain fork (Fig. 31) or arm (Fig. 32). This is a most convenient stand, with a long working distance.

Most dissecting microscopes are, however, simple lenses of the aplanatic or less expensive type, mounted either in a holder or on a stand, with various forms of

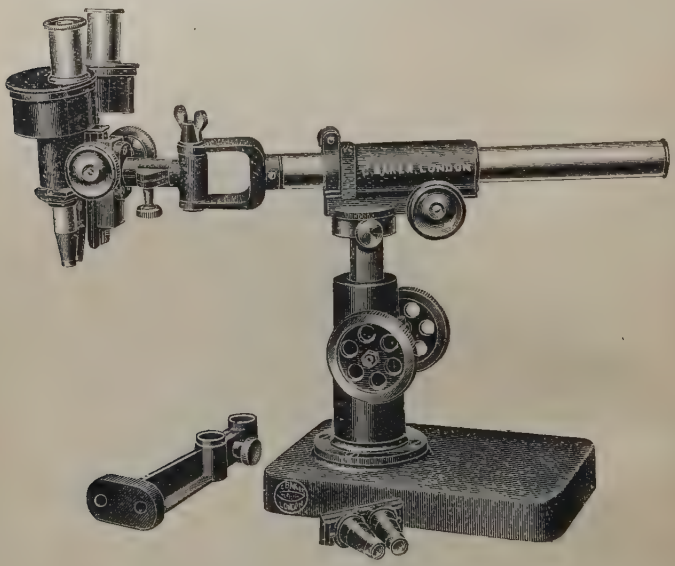


FIG. 32.

movable arms giving universal movements (Fig. 33). Wherever possible an aplanatic triplet, as described on p. 5, should be selected, its relatively large working distance and perfect corrections saving the eye much fatigue in prolonged work. Until recently the Continental makers showed a decided superiority over our own makers in the stands to hold these lenses, both in design and in cheapness, combined with good work; but English

and American firms now make excellent dissecting stands, two of which are illustrated. The first is a simple dis-

FIG. 33.—MAGNIFIER HOLDERS.

secting stage with opal glass plate and movable lens-holder (Fig. 34). The second (Fig. 35) is an upright stand

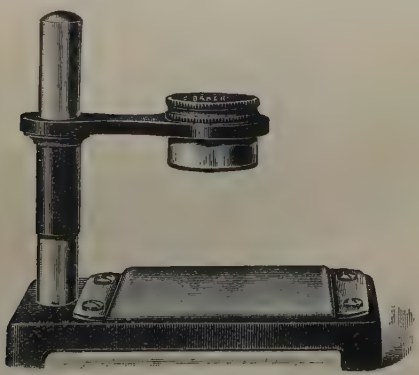


FIG. 34.—SIMPLE DISSECTING STAND.

bearing a square glass stage, provided with springs, lens-holder adjustable by a screw, movable plane mirror and

opal glass reflector, and movable hand-rests. The addition of a Porro erecting prism (Fig. 36) to this type of

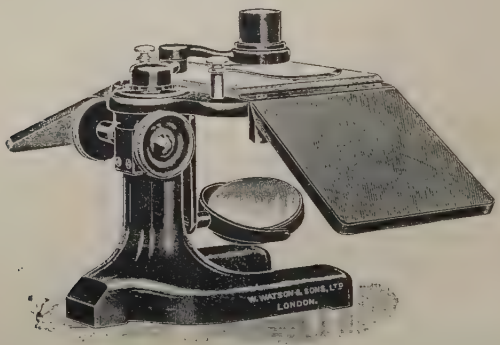


FIG. 35.—DISSECTING STAND.

dissecting stand enables ordinary objectives to be used, and gives an erect image and greater working distance.

FIG. 36.—'PORRO' ERECTING PRISM.

For ordinary work this pattern is very convenient. Another good form of inexpensive dissecting stand is made by Bausch and Lomb. It is cut out of a solid

block of wood, shaped so as to form hand-rests. The glass stage is extra large, and is easily removed for cleaning. The mirror is rectangular, and is as large as

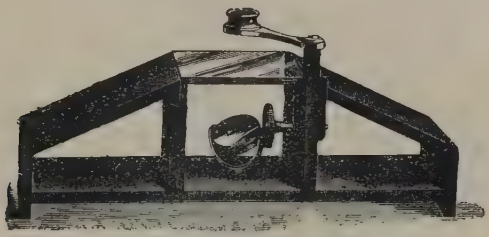


FIG. 37.—SIMPLE DISSECTING STAND.

the stage, being set at an angle beneath it. A plate, with a black and white side, is stowed beneath the stand, and may be laid over the mirror to form a black

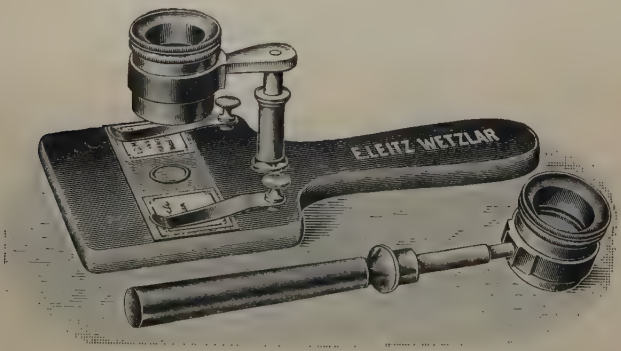


FIG. 38.—'DEMONSTRATION' LENS-HOLDER.

or white background as desired. The lens-carrier slides in a metal sleeve, which is firmly fixed in the wooden base. Similar inexpensive stands are made by Watson and other makers (Fig. 37), and these can even be made

at home, using an inclinable square piece of mirror beneath the stage, and any kind of lens-holder above it.

Lens-Holders.—Most of our English makers have simple holders for lenses at varying prices, as already stated. Various types of demonstration microscopes

FIG. 39.—'DEMONSTRATION' MICROSCOPE.

for classes are also sold, varying from a simple handle holding a microscopic slide, and an aplanatic lens above it (Fig. 38), to a more elaborate tube carrying eyepiece and objective within the body, the slide being supported by springs on a plate at the end (Fig. 39). Either of these can readily be handed round a class.

Tank Microscope.—Finally, we may mention the Tank microscope (Fig. 40), designed by Mr. C. F. Rousselet, for rapidly looking over pond-water, weeds, etc. It is made by C. Baker and by Beck, and consists of a tank $7 \times 5 \times 1$ inches, standing on edge, on a broad base, to which is added an upright with a jointed arm carrying an aplanatic lens of long focus, say $\times$ 8, which can be

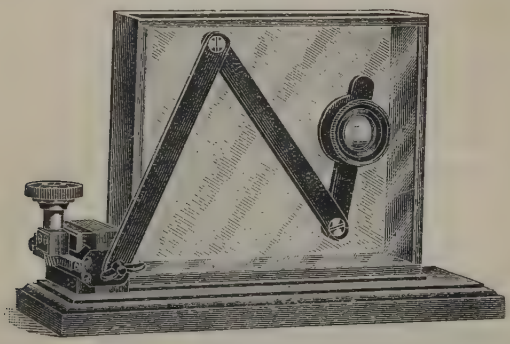


FIG. 40.—TANK MICROSCOPE.

moved to any part of the tank, and is adjustable by rack and pinion. The latter can be detached and clamped to another base for use as a dissecting microscope.

Microscopes for Special Purposes.—Before leaving the subject it may be useful to mention certain instruments which have special uses. Both Leitz and Reichert make microscopes with very large stages of special construction, about 8 inches square, by means of which large sections, such as those of brain, can be conveniently examined, whilst a special traversing microscope made by Leitz is worth illustrating (Fig. 41), and explains itself. The range of motion of this microscope is over 7 inches, and of the stage over $5\frac{1}{4}$ inches. It is very

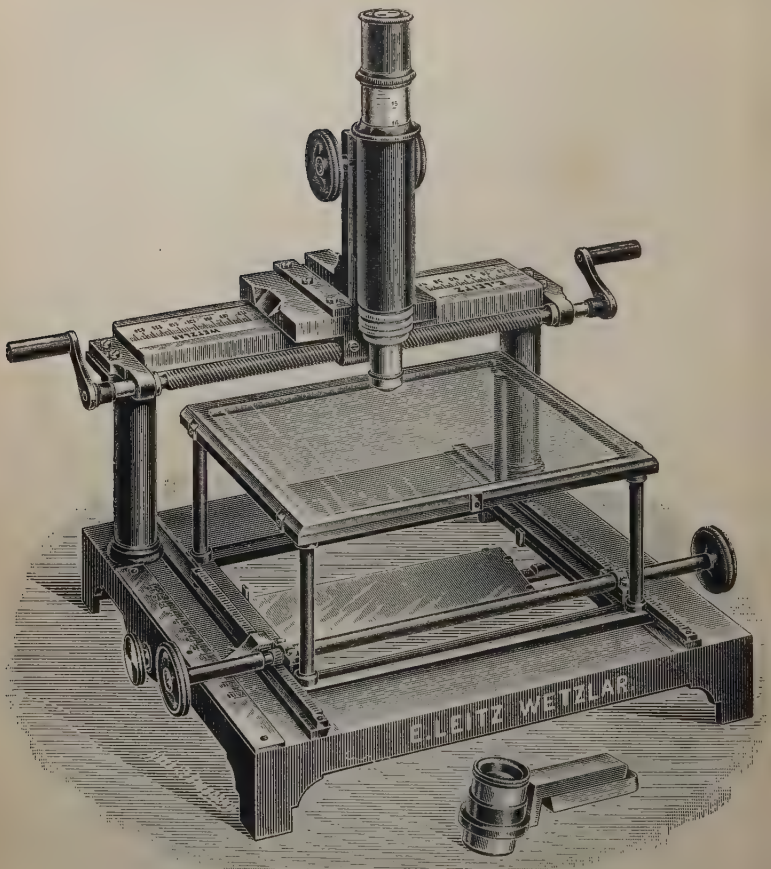


FIG. 41.—LEITZ'S TRAVERSING MICROSCOPE.

convenient for searching large dishes. Leitz and Watson also make special museum microscopes in which a large drum, carrying a number of slides, is rotated beneath the microscopes, but these are manifestly of limited application.

Projection Microscopes.—The ordinary microscope is not suited for lantern projection on a screen, as it contains unnecessary elaborations, is fitted with too narrow and too long a tube, and does not adapt itself readily to

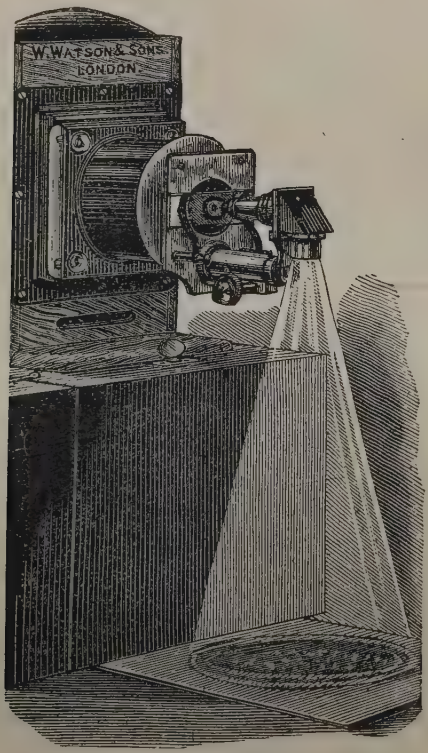


FIG. 42.—PROJECTION MICROSCOPE.

enclosing the source of illumination. The accompanying illustration (Fig. 42) shows a simple attachment for an ordinary lantern made for the writer many years ago. It replaces the projection lens of the lantern, and screws

into its ring, and contains an adjustable plano-concave lens for focussing the light on the object. The remainder of the apparatus consists of a simple microscope stage with springs and a rotating wheel of diaphragms, and a short limb with coarse adjustment, carrying a mere nose-piece without body or ocular. Ordinary objectives are used. The loss of light is so great that it is seldom worth while using even moderately high-power objectives for lantern projection, therefore this simple arrangement fulfils most requirements. Finally a right-angle prism, readily swung aside, is provided, so that the apparatus can be used for drawing upon the table (see p. 71), or for demonstration in this position to a small class of students. A glass trough filled with alum water should be placed near the main condensing lens of the lantern in the space provided for lantern-slides, as the heat may damage either preparations or objectives if used for any length of time with a powerful illuminant.

With the more elaborate forms of projection apparatus it is not within the province of this book to deal.

CHAPTER IV

OBJECTIVES AND EYEPIECES

Achromatic Objectives.—It now becomes necessary to speak of the purely optical parts of the microscope, the most important of which are, of course, the objectives. The improvement in objectives of late years has been enormous, and for a reasonable sum the microscopist can now get lenses with a definition that leaves but little to be desired. This is largely due to the use of the newer forms of glass, known generally as Jena glasses, but also to entirely new computations by means of which aberrations have been largely reduced. This is evident not only in the clearness and brightness of the image, as well as in its definition, but also in the increased ability of the objective to bear high eyepiecing without an immediate loss of definition. It is a mistake, however, to suppose, as is so often stated, that *any* objective will bear very high eyepiecing without depreciation in the image, and this is most evident with the high powers, but many of the later achromatics will now stand *moderate* eyepiecing as well as the nearly apochromatics. In fact, we have had in our hands achromatic objectives which, except that the chromatic corrections are necessarily less perfect, would compare favourably in definition, in brightness of image, and in power to bear high eyepiecing with the apochromatics. This is the more important as the price of these latter lenses is almost prohibitive to workers of moderate means, a $\frac{1}{12}$-inch oil-immersion costing £12 to £20,

according to its aperture. The reader may rest assured, therefore, that good achromatics, by first-class makers, will show him *almost* all that the microscope is capable of revealing.

Rating of Objectives.—In Chapter VI. it will be explained (see p. 140) that the terms 1 inch, $\frac{1}{4}$ inch, etc., as applied to objectives, do not represent the distance between the under side of the front lens and the object, but represent the equivalent focal length, and therefore the approximate initial magnifying powers without eyepiece at a distance of 10 inches from the back lens.* Owing to the difficulty of making objectives of high aperture and proportionately low magnifying power, objectives will often be found to be of higher magnification than they should be. An inch will be nearer $\frac{3}{4}$ inch, $\frac{1}{2}$ inch nearer $\frac{1}{3}$ inch, and so on. The *testing of objectives* requires much practice and education of the eye.

Choice of Objectives.—With regard to the choice of individual objectives by different makers, we strongly recommend the beginner to get his objectives only from the very foremost makers. Their selection is a most important matter, and it is here that the advice of a really competent friend becomes valuable. When all is said and done, the objective is, after all, the most important part of the microscope. Microscopes are often

* Strictly speaking, it should be 10 inches from the upper focal plane of the objective; but this is not easily found in a modern compound objective, and the above is near enough for all practical purposes. The objective may be corrected for, and intended to be used with, a tube-length of $6\frac{1}{2}$ inches only, as is now so customary, in which case the initial magnification will be lower in this proportion, but the above uniform system of rating is adhered to in order to save confusion. Ten inches is selected because it is the normal visual distance for distinct vision. (See p. 146.)

quoted with a 1-inch and a $\frac{1}{4}$-inch or $\frac{1}{6}$-inch objective. It generally pays to purchase the objectives, where possible, or at any rate some of them, from the maker of the microscope, as several makers charge a little less in such cases than if the items are bought separately. In fact, certain makers do not sell their stands alone.

Apochromatic Objectives.—The apochromatics are high in price, but there is no doubt of their superiority to other objectives, and those who propose to do critical and original research work will purchase them accordingly. Their advantages are wide apertures, and therefore maximum powers of resolution, freedom from colour, brighter illumination, and greater capacity to stand high eyepiecing. They are the best of all lenses for photo-micrography without screens, but when monochromatic screens are used, their superiority over the achromatics is much less evident. Their visual and actinic foci coincide. It must be added, however, that to get the best out of an apochromatic requires both knowledge and practice, and therefore in unskilled hands they may show little superiority over the achromatics. They must always be used with compensating eyepieces, and are somewhat round in the field. Messrs. Zeiss, owing to the prestige gained by Professor Abbe's investigations, and to the fact that they first put these lenses upon the market, are perhaps the largest makers of apochromatic lenses, and are in consequence able to turn them out with exceptional uniformity. Whether their high price is justified is another matter. As dry lenses they make a $\frac{2}{3}$-inch and $\frac{1}{3}$-inch of N.A. 0·3 (the former is now made to order only); a $\frac{1}{2}$-inch and $\frac{1}{3}$-inch of N.A. 0·65; and a $\frac{1}{4}$-inch, $\frac{1}{6}$-inch, and $\frac{1}{8}$-inch of N.A. 0·95. They make also a water-immersion $\frac{1}{10}$-inch of 1·25 N.A., and oil-immersions $\frac{1}{8}$-inch and $\frac{1}{12}$-inch of N.A.

1·3, and a $\frac{1}{8}$-inch and $\frac{1}{12}$-inch of N.A. 1·4. All these are made either for the short or long tube as required, with the exception of the 1-inch, the $\frac{1}{2}$-inch, and the $\frac{1}{4}$-inch, which are made for the long tube and to order only. It will be noticed that the N.A. of these lenses is exceptionally high, the $\frac{1}{4}$-inch in particular being a most difficult lens to construct. The $\frac{1}{2}$-inch of 0·65 N.A. is considered the finest dry lens on the market, and the $\frac{1}{8}$-inch oil-immersion of 1·4 N.A. is considered equally the finest immersion lens. A complete battery for an advanced worker with a deep enough pocket might be taken as including the 1-inch, the $\frac{1}{2}$-inch, the $\frac{1}{4}$-inch, the $\frac{1}{8}$-inch of the higher aperture, and the $\frac{1}{12}$-inch of the lower aperture, all corrected for the 10-inch tube. The $\frac{1}{6}$-inch might be recommended in preference to the $\frac{1}{4}$-inch as being more generally useful, and as being more uniform in quality. For the now almost universal short tube the $\frac{2}{3}$, $\frac{1}{3}$, $\frac{1}{6}$, and $\frac{1}{12}$ inch would be the most useful, but the ratio of aperture to power is, of course, lower, and the initial magnification less (see p. 142). The lower aperture of 1·3 N.A. is recommended for the $\frac{1}{12}$-inch in preference to that of 1·4 N.A., not so much because it is less in price, but because, as the makers state, the resolving and defining power of the latter is only 8 per cent., and the brightness of its image only 14 per cent., more than those of the lower apertured lens of the same power, whilst the more than hemispherical form of the front lens makes it difficult to mount, and as a result it is easily damaged or displaced. The shorter working distance, 0·2 millimetre as against 0·25 millimetre, constitutes another drawback to its use, unless the maximum of resolution is imperatively required. Zeiss actually makes (to order only) a $\frac{1}{10}$-inch monobromide of naphthalene immersion lens with the extraordinary N.A.

of 1·6 (proportionate to the refractive index of this medium), which has been used by the late Dr. Henri Van Heurck, of Antwerp, for his exquisite photographs of diatom structure; but it necessitates the use of special cover-glasses and mounting medium of the same high refractive index, and a medium has not yet been found which could be used for objects other than diatoms without destroying either their structure or colour, or damaging the front lens of the objective. This drawback militates against the wider use of the objective, whilst the price is not less than £40.

The late firm of Powell and Lealand lost no time in manufacturing apochromatic objectives according to the new formulæ, and their lenses were stated by competent observers to be not inferior to those of Zeiss, though possibly a shade less uniform, and excellent apochromatic objectives are now made by most of the leading English, American, and Continental makers.

Semi-Apochromatic Objectives.—The most notable advance in recent years is, however, the improvement in achromatics, due largely to the use of the new Jena glasses, and to this we have previously alluded. Such objectives are often spoken of as semi-apochromatics, or by trade designations, and though one may object on principle to the multiplication of descriptive names, the more recent lenses almost deserve some such title to distinguish them from the earlier achromatics, for they run even the apochromatics very close, and except for a certain slight amount of outstanding colour on critical tests, they are not markedly inferior to the best of the apochromatics, and bear high eyepiecing almost equally well.

Achromatic Objectives.—In ordinary achromatic objectives, of what is generally known as the students'

series, there is a wide choice, but it is worth noticing that each maker has one or more objectives which are specially good.

All achromatic lenses are now corrected for the short tube, unless specially ordered otherwise.

The best all-round objective for the beginner is the 1-inch or $\frac{2}{3}$-inch, after that the $\frac{1}{4}$-inch or $\frac{1}{6}$-inch—the former preferably for botanical work, and the latter for human histology. As the worker becomes further

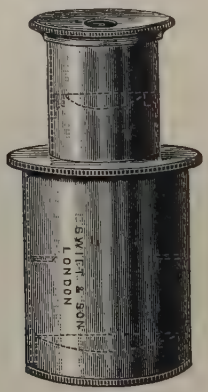


FIG. 43.—HUYGHENIAN OCULAR (SECTION).

advanced, he will wish to add others. The 2-inch is a most useful lens, as it takes in a large field; the $\frac{1}{2}$-inch comes in between the 1-inch and the $\frac{1}{4}$-inch, and a $\frac{1}{8}$-inch (dry) is useful in certain circumstances. Any higher power than this should be an immersion lens, preferably oil-immersion. But the beginner is not advised to get really high powers until he actually requires them. He will soon realize that mere magnification is by no means the main object in microscopical work, and that the real worker invariably uses the lowest power he can. Above

all things, he should remember that the homogeneous immersion objectives are greatly superior in resolution, brightness of illumination, working distance, and in relative insensibility to disturbance of correction due to variation in cover-glass thickness, to dry lenses of equal magnification, and unless the use of a dry lens is imperative for some reason, a $\frac{1}{12}$-inch should always be an immersion lens. The $\frac{1}{6}$-inch will generally prove as efficient as a $\frac{1}{8}$-inch, and be much easier to work with. Therefore really high power *dry* lenses should be avoided, if possible.

Tank Objectives.—These are specially constructed and corrected for the examination of objectives in a tank or receptacle of water, and are usually about 1 inch and $\frac{1}{2}$ inch equivalent focal length.

Oculars.—The necessary **eyepieces** must now be dealt with, and on this matter fewer words will be necessary than have been advisable with regard to objectives. The eyepiece most frequently met with is the **'Huyghenian'** or **'negative' eyepiece** (Fig. 43), which is composed of two plano-convex lenses with a diaphragm in the principal focus of the eye-lens, the principle being explained on p. 142. The diaphragm cuts off the marginal rays, which suffer most from spherical aberration, and the field-lens in this type of eyepiece assists in correcting the aberrations of the objective, and so tends to flatten the field.

The **Kellner Eyepiece** (Fig. 44) is variously stated as both a positive and a negative eyepiece, the fact being that the field-lens is *in* the focus of the eye-lens. This field-lens is biconvex, whilst the eye-lens is an achromatic doublet meniscus. The field is only bounded by the diameter of the tube, and is therefore proportionately large—in fact, in a microscope with a large tube and

correspondingly large eyepiece it may take in a field larger than is advisable, in consideration of the increasing marginal aberrations of the objective. Its definition is not quite so good as that of the negative eyepiece, and it shows dust on the field-lens readily, but for low-power work its large field makes it often useful.

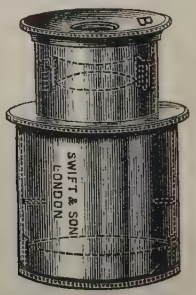


FIG. 44.—KELLNER OCULAR (SECTION).

The **Ramsden Eyepiece** is really a positive eyepiece which means that its focus is slightly outside the field-lens, and it can therefore be used as a magnifying-glass. It consists of an achromatic combination of two plano-convex lenses, with their plane sides outwards. These oculars were formerly used as micrometer eyepieces, the micrometer divisions being readily magnified by this means, but the micrometer is now generally placed in the position of the diaphragm in a Huyghenian eyepiece.

Compensating Oculars.—The **Compensating Eyepieces** of Zeiss and other makers are specially constructed for use with apochromatic objectives, in which, as hereafter explained (p. 135), certain outstanding corrections are deliberately left to be rectified by the eyepiece. The apochromatic objectives being left under-corrected,

the compensating eyepieces are made correspondingly *over*-corrected. Strictly speaking, it was only the high-power apochromatic objectives that required to be left under-corrected in this way; but, to permit of the use of these eyepieces throughout, the same under-correction is put into the low-power apochromatic objectives also. These eyepieces are made with initial magnifications stated as 3, 5, 7, 10, 15, and 25, the first three being Huyghenian eyepieces with achromatic eye-lenses; but the powers from 8 upwards are positive, containing an achromatic double convex triplet with a single plano-

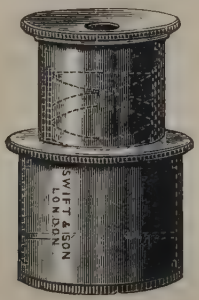


FIG. 45.—COMPENSATING OCULAR (SECTION).

convex lens above it (Fig. 45). The field is limited to 6 inches, for the reasons mentioned when dealing with Kellner eyepieces. The compensating eyepieces are all arranged to work in the same focal plane, about 12 millimetres below the top of the tube, and the necessary re-focussing after a change of eyepiece is thus reduced to a minimum, whilst the corrections of the objective are not disturbed. The initial magnifications of the eyepieces are marked upon them and represent the number of times with observers of normal vision that they magnify the image formed by the objective, which will

itself depend upon its focal length and the tube-length at which it works. Formerly they were marked with numbers 40 per cent. less than their actual powers of magnification, based on the combined magnification, which would otherwise have diminished, owing to the objective no longer forming its image at 10 inches, but at 6. In other words, with the short tube the objective gives 40 per cent. less magnification than with the long tube, but the total magnification was kept up by increasing the magnification of the ocular and marking it down as if the objective still gave the same magnification as with the 10-inch tube. It led to much confusion and misunderstanding, and Continental opticians, and unfortunately not a few English opticians also, persisted in giving in their catalogues tables of combined magnification in which the objectives corrected for the short tube-length were treated as if they were intended to be used at the full 10-inch magnification, and the powers of the eyepieces were correspondingly understated. In the previous edition of this book a strong protest was made against this misleading system, and it is a satisfaction to note that the initial magnification of the ocular is now treated as an independent and unvarying quantity, and the magnification of an objective admitted to depend upon the distance at which it is designed to form its image.

Before leaving the compensating eyepieces, we may mention that, owing to the fact that ordinary high-power achromatic objectives have errors leading to under-correction, the compensating eyepieces distinctly improve the performance of such objectives.

Universal Oculars.—W. Watson and Sons have introduced adjustable negative eyepieces, made of denser glass with higher separation, in which the eye-lens is

mounted in a short draw-tube within the tube carrying the field-lens, this tube being capable of an adjustment of about 10 millimetres (Fig. 46). The side of the draw-tube is graduated accordingly. When the inner tube is pushed home the eyepiece magnifies all colours equally, and is suitable for use with low-power achromatic objectives (which are generally over-corrected), but when the inner tube is drawn out the eyepiece magnifies red more

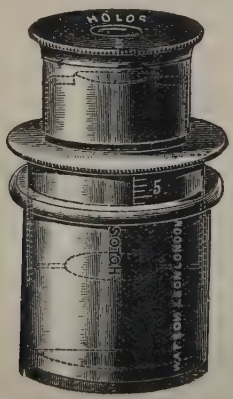


FIG. 46.—WATSON'S 'HOLOSCOPIC' OCULAR (SECTION).

than blue, and serves as a compensating eyepiece for use with all apochromatic and with high-power achromatic objectives, as above stated.*

Eyepieces are made either in the students' form, or with caps upon them. The capped eyepieces are used mainly for the larger English stands. The students' type of eyepiece is less expensive than the compensating eyepiece.

* A convenient method of distinguishing an ordinary Huyghenian eyepiece from a compensating one is to look through it at a white light. The former will show a bluish edge round the diaphragm, whilst the latter will show a yellowish-red one.

Choice of Oculars.—The magnifications of eyepieces range from 5 to 15, or even higher. In choosing them, it should be borne in mind that an initial magnification of 5 or 6 is about the lowest that can be satisfactorily used on the short tube-length, for which nearly all the achromatic lenses mentioned in this chapter are corrected, and that, on the other hand, few achromatic objectives, even of low powers, will satisfactorily bear really high eyepiecing. We are quite aware that all makers sell eyepieces of much higher powers, as mentioned above, but very few of the ordinary achromatic objectives will satisfactorily and *critically* bear eyepiecing above ten times with a 10-inch tube-length. It is only the apochromatics that will bear very high eyepiecing, and even in their case the loss of light and depreciation of the image is noticeable (see p. 156). Moreover, high-power eyepieces have necessarily a small eye-point diameter, and any dust present becomes very obvious in the image. But until the apochromatics were invented the only way to get high magnification was to use high-power objectives, such as $\frac{1}{25}$- and $\frac{1}{50}$-inch, which we so seldom hear of nowadays. If the microscope be provided with two eyepieces, therefore, we would suggest that the 6 and 10 should be chosen. They should all work in about the same focal plane to minimize the necessary re-focussing, should fit easily in the draw-tube, in order to obviate any risk of forcing the objective down on the cover-glass, and should be marked with their actual magnifications, instead of arbitrary figures or letters.

Projection Ocular.—There are certain eyepieces that are used for special purposes. Amongst these is the **Projection Eyepiece** (Fig. 47), used with the photo-micrographic camera, and for other projection purposes. It possesses a collective field-lens and an achromatic

biconvex triplet eye-lens. This eyepiece should be free from secondary chromatic aberrations, and the visual and actinic foci should coincide. A limiting diaphragm is placed between the lenses, which must be focussed upon the screen used. These eyepieces are over-corrected, like the compensating eyepieces, and are intended to be used with high-power achromatic objectives. They give beautiful definition for projection purposes, and are necessary for the highest class of photo-micrography, though the compensating oculars, and with low powers

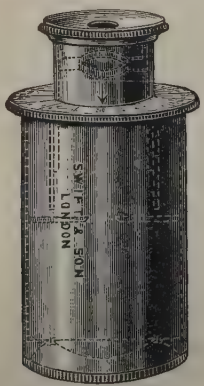


FIG. 47.—PROJECTION OCULAR (SECTION).

even the ordinary negative eyepiece, give good results, but with high powers the latter, constructed to form a virtual image in the microscope, do not give a flat field when focussed outside the microscope. The projection oculars are made in powers of 3, 6, and 8, and can be used either for the 10-inch or the 6-inch tube. In the latter case they are sometimes misdescribed as magnifying 2 and 4 times respectively.

Double Demonstration Eyepiece.—This is a tube set at right angles to the eyepiece which enables observers

to view the image simultaneously. It contains a reflecting prism and adjustment for difference of vision. It is provided with a pointer in the plane of the diaphragm coinciding with that of the image. It is useful also for watching the movements of bacteria for photography with dark-ground illumination, in order to select the right moment for exposure.

Micrometer Eyepiece.—The **Micrometer Eyepiece,** as well as spectroscopic and polarizing eyepieces, will be dealt with under accessories.

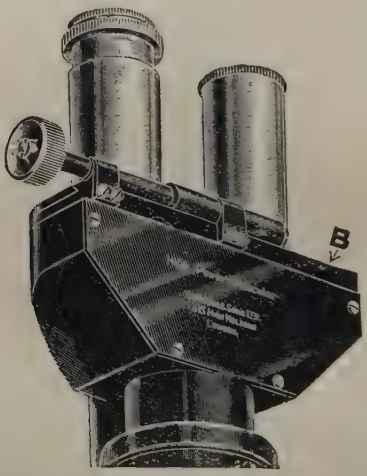


FIG. 48.—BINOCULAR EYEPIECE.

The **Binocular Eyepiece**, which must be sharply distinguished from the binocular microscope of the earlier Wenham type, or its later modifications (see pp. 52 and 61), and from the Greenough type of microscope with two objectives, is illustrated in Figs. 48 and 49, and can be used with the highest powers. The principle is that of a

half silvered cemented surface in the middle prism, allowing half the rays to pass, and deflecting the other half. The two oculars are generally placed parallel to each other, though the Spencer Lens Company's design, in which the tubes are set at an angle so as to follow the convergence of the eyes, seems preferable.

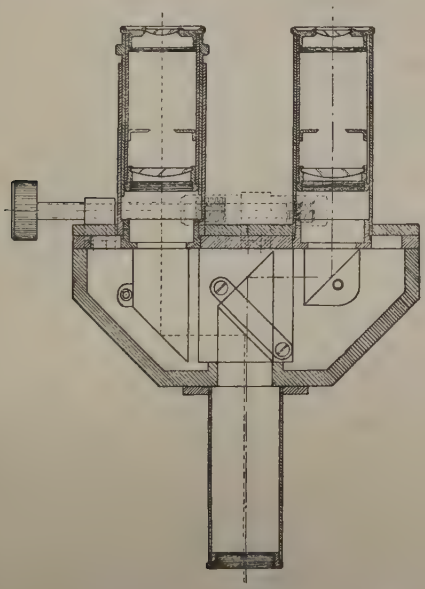


FIG. 49.—BINOCULAR EYEPIECE.

They are adjustable for inter-pupillary width, and one ocular can be focussed if there should be a difference between the eyes. The resulting image is very comfortable to work with, using both eyes equally, and requiring no practice, but the stereoscopic or pseudo-stereoscopic effect does not yield the highest resolution. There is very considerable loss of light, and for the

highest class of critical work the definition suffers. Two other drawbacks are that the increased length of path travelled by the rays from the object give much greater magnification and a correspondingly smaller field. To rectify this a negative lens is generally fitted at the lower part of the attachment, and low-power eyepieces are used, or the eyepieces are mounted separately. The other drawback is that the ocular attachment is heavy, and puts a proportional strain upon the fine adjustment. For this reason it is now more commonly sold as a binocular microscope than as an eyepiece attachment (see Chapter III.).

A list of the standard eyepiece sizes has been given on p. 26, and in the Appendix, and no microscope should be purchased which does not conform to one of these.

CHAPTER V

ACCESSORIES

Sub-Stage Condenser.—The **Sub-Stage Condenser** is now admitted to be an essential portion of the equipment of the modern microscope. This valuable adjunct has, indeed, been long recognized by English microscopists and opticians to be indispensable, but it took many years before it received due appreciation on the Continent. The majority of microscopical objects are examined by transmitted light, and the condenser serves to focus the light upon the object and to control it. As the qualities of modern objectives have advanced, so the condenser has been improved to keep pace with them, until now it is an optical masterpiece, second only in its corrections to the objective; and upon the perfection of these corrections much of the performance of a high-class objective on critical work depends.

Types of Condenser.—The present-day condenser is mainly of two types—one *chromatic*, known as the 'Abbe illuminator,' and one *achromatic*. The former is the most popular, the cheapest, and the most frequently fitted to students' microscopes both in this country and abroad, but the superiority of the latter is unquestioned.

Abbe Illuminator.—The **Abbe Illuminator** is of two types (Figs. 50 and 51), a double combination with an aperture of 1·2 N.A. and a triple form of 1·4 N.A. Of course, when the whole of this aperture is required the condenser must be in immersion contact with the

under side of the object slide. Both are simple, cheap, and easy to work, and are accordingly largely used by students; but in both the chromatic and spherical aberrations are very great, and they are therefore not satisfactory when used for critical work. The value of a condenser depends upon the **aplanatic cone** (see

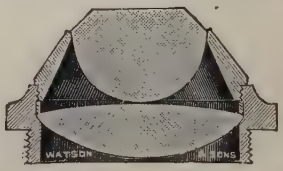


FIG. 50.—ABBE ILLUMINATOR, N.A. 1·2 (SECTION).

p. 160) which it is capable of transmitting, and this, in the two condensers under discussion, is only about 0·5 N.A. At the same time, as we have just said, the beginner will find it a very useful form of condenser for general and not too critical work. We prefer the 1·2 N.A. type, and it is also the cheaper of the two.

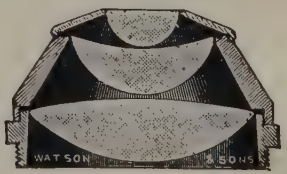


FIG. 51.—ABBE ILLUMINATOR, N.A. 1·4 (SECTION).

Achromatic Condensers.—The **Achromatic Condenser** of 1·0 N.A., made by most English and one or two Continental opticians, is much superior to the Abbe illuminator, not only because it is achromatic, but because it transmits an aplanatic cone approximating to its N.A., though this last is nominally less than in the chromatic forms. Its advantages are especially noticeable in photo-

micrography. Until recently it was somewhat heavy and cumbrous in shape, so that in many microscopes it had a tendency to foul the sliding-bar or mechanical adjustments of the stage. The later condensers of most makers are compact in size and excellent in every way. As a type of these may be mentioned Watson's 'Universal' Condenser (Fig. 52), which has a power of 0·4 inch with N.A. 1·0, but is capable of transmitting an aplanatic cone exceeding 0·95. This is a useful condenser for moderate and high powers, and can be used with lower powers by removing the top lens, when the power becomes 1 inch; but the N.A. drops proportionately to 0·4 N.A., and a certain amount of chromatic over-

FIG. 52.—WATSON'S 'UNIVERSAL' CONDENSER (SECTION).

correction becomes noticeable. The same makers and others have recently brought out special condensers for use with low powers, which are useful where the whole field is required to be evenly illuminated, as in photo-micrography. Condensers of about $\frac{1}{4}$-inch power are also made, for special use with moderately high-powered objectives.

Finally may be mentioned the **oil-immersion condensers,** with large N.A. and magnifying power of about $\frac{1}{2}$ inch, which are essential if the extreme resolving and defining powers of immersion objectives are to be realized (see p. 175). Of these Watson's immersion condenser may be taken as an example (Fig. 53), having a N.A. of

1·3 to 1·4, and an aplanatic cone of nearly the same, varying with the thickness of slip for which it has been adjusted to work, a greater working distance reducing the N.A. As usually adjusted for slip 1·6 millimetres thick, it has a N.A. of 1·34. With the top lens removed and used dry it makes an efficient condenser of about $\frac{1}{2}$-inch magnification and aplanatic aperture of 0·55 for use with lower-powered objectives.

It will be understood that these high-powered condensers give a smaller field of illumination, but a correspondingly more concentrated light.

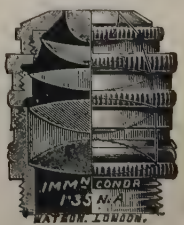


FIG. 53.—WATSON'S IMMERSION CONDENSER (SECTION).

For all ordinary purposes the moderate-powered condensers, such as Watson's 'Universal,' will be found most generally useful.

It may be added that the top lens of all condensers is generally removable so as to give a larger illuminated field when a low-power objective is being used. The mount (Fig. 54) of the condenser is either carried by the focussing and centring stage mentioned when we dealt with stands, or it slides in a short tube-ring affixed to the under side of the stage. Of course, the former is much preferable, not only in the facilities it gives for focussing, but also for centring, though at least one maker provides a centring but non-focussing sub-stage ring, which can also be readily swung aside when not wanted. Beneath

the condenser is fitted an iris diaphragm, or a ring to hold movable diaphragms, and there is generally also an additional turn-out ring into which stops can be placed to give oblique illumination (necessary at times for certain diatoms and similar work), to hold coloured glasses, or to give dark-ground illumination by means of a central black spot, varying in size with the objective used (see p. 178). These give most beautiful effects, but are not often used for critical work.

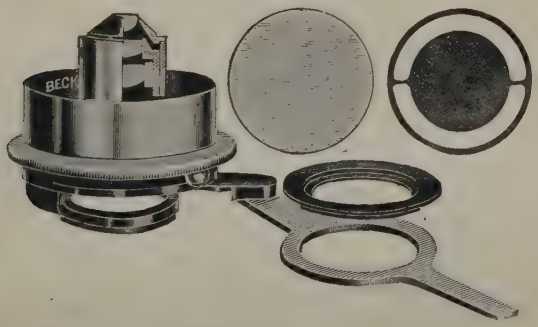


FIG. 54.—CONDENSER MOUNT AND FITTINGS.

Dark-Ground Illumination.—A beautifully made expanding stop is now made, known as the 'Traviss' Expanding Stop (Fig. 55), by which exact adjustment can be readily obtained, the variation being from $\frac{4}{10}$ to $\frac{3}{4}$, or even to $\frac{9}{10}$ of an inch. It is, unfortunately, somewhat delicate in construction, and therefore liable to suffer if too carelessly treated.

Dark-ground illumination can also be obtained by a stop behind the objective, but this is of less general application.

The **spot-lens** proper and the **paraboloid** were very popular in this country a good many years ago, but fell

into disuse until revived as new on the Continent, and they are now sold by all makers for illuminating unstained bacteria examined with high-power ($\frac{1}{8}$- and $\frac{1}{12}$-inch) objectives. The construction of these paraboloidal condensers varies somewhat, but they are all

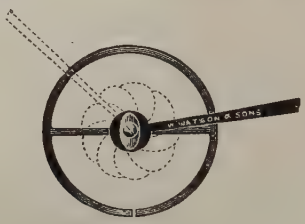


FIG. 55.—'TRAVISS' EXPANDING STOP.

immersion condensers of high aperture, and in all the central rays of light are blocked out by a stop, which cuts out direct light of less than N.A. 1·0 to 1·1, whilst the marginal rays of higher aperture are reflected from the paraboloidal sides of the condenser (Fig. 56), and refracted by the object, all other rays than those thus

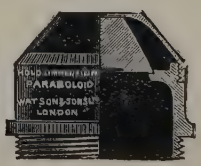


FIG. 56.—IMMERSION PARABOLOID.

refracted being totally reflected by the cover-glass. Thus only the light which is refracted by the object enters the objective, and the object shows up illuminated on a black or dark background. In the newer forms there are two internal reflections, as shown in Fig. 57, with freedom from spherical aberration, a sharper focus,

more light and more perfect dark ground, but greater sensitiveness to errors of adjustment, variations in thickness of slide, dust, etc. Strong parallel light is necessary, preferably from a small arc lamp, and the object must be mounted in water or oil, *whilst the slide must be in immersion contact with the condenser.* It is to be noted that to get perfect dark-ground illumination the objectives must be medium or high power objectives of not more than 1·0 N.A., immersion lenses being specially stopped down to this aperture, and the slide must be of a definite thickness, generally about 1 millimetre, marked by each maker on his condenser, and varying not more than 0·1 millimetre each way. The cover-glasses also must be of a definite thickness, generally 0·17 millimetre, and careful centring of the condenser is necessary. Oil-bubbles, dust, etc., disturb the effect, and the slide, the liquid medium, and the cover-glass must all be parallel, or uneven diffraction effects will result. It will be noted, therefore, that no little attention to detail is required if satisfactory results are to be obtained, and the adjustment of the light and centring of the condenser require a certain amount of practice (see p. 176). Most makers supply printed instructions on these points with their particular design of **dark-ground immersion condenser.** The effects are, of course, diffraction effects, and the resulting image, though very beautiful, is not in any sense a critical one, and therefore too much should not be expected from these condensers in investigating structure. They have an advantage over the ordinary stop beneath the condenser in being free from chromatic dispersion, as the light is reflected, and not refracted.

Beck has modified this type of condenser so that it can be adjusted for variations in thickness of slide—namely, from 0·5 to 1·5 millimetres (Fig. 57). When

living bacteria are examined with these condensers by means of immersion lenses, the result is very striking, and the bacteria are much better shown than by the customary 'hanging drop' method, with nearly closed and lowered diaphragm.

It will be noted that in the systems described the object is lit up by rays of light exceeding 1 N.A., and that the objective (if an immersion lens) has to be cut down so that its aperture does not exceed 1 N.A., thus reducing

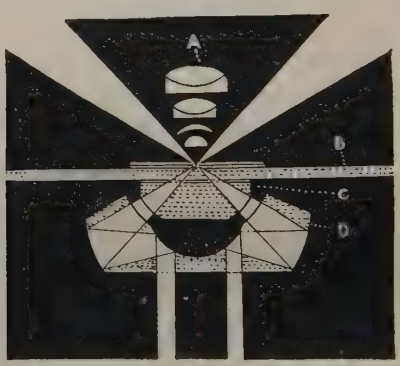


FIG. 57.—BECK'S FOCUSSING DARK-GROUND IMMERSION CONDENSER (SECTION).

the efficiency of the lens by about 25 per cent. Watson and Sons now make a 'Cassegrain' oil-immersion dark-ground illuminator, designed by Mr. E. M. Nelson (Fig. 58), which will work with any $\frac{1}{12}$ objective up to 1·3 N.A., and with a slip varying from 1 to 1·2 millimetres thick, and which requires less powerful illumination. It is provided with small removable central discs to get the best effect.

Mention should be made here of the ingenious colour-screens of Mr. Julius Rheinberg, which consist of discs of

gelatine, inserted in the ring beneath the condenser, and of which the centre of each disc is of a different colour from the periphery. The results are unexpectedly beautiful, the object and background being differently coloured. They can be bought in various colours, and the writer has made them from ordinary sheets of coloured gelatine. By the use of suitable screens or light filters in cases where the preparation is stained with a dye having a small band of absorption in the spectrum, in conjunction with a dark-ground condenser, increased contrast can be obtained on a dark field, as in tubercle bacilli

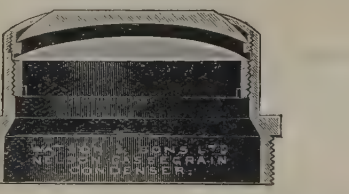


FIG. 58.—NELSON'S CASSEGRAIN IMMERSION PARABOLOID (SECTION).

stained as usual with methyl-blue and carbol-fuchsin (Ziehl-Neelsen method), and a spectroscopically pure blue filter for the illuminant, the bacilli showing up alone on a dark field.

Diaphragms.—An **Iris Diaphragm** is greatly superior in convenience at least to any form of **diaphragm plate,** whether it be a revolving plate or a series of stops. Under any circumstances the proper place for the diaphragm is beneath the condenser. Certain American and Continental, and one or two English, stands provide two diaphragms, one beneath the condenser and attached to it, and one above for use when the condenser is removed. These microscopes also contain an ingenious

refinement, already alluded to (p. 45), by means of which the diaphragm can be racked backwards and forwards in a plane at right angles to the optic axis. In diatom work this is of service, and also for lens-testing, but for all general purposes it seems to us to be as unnecessary as it is cumbrous and costly. It is certainly not rigid, and as the Continental sub-stage is generally guiltless of centring screws, it appears to be misplaced ingenuity to provide an elaborate means of *decentring* the diaphragm, which is seldom needed, without making any provision for the optical *centring* that is so essential in critical work. Watson supplies this form of lateral adjustment to the diaphragm as part of the condenser mount, which will fit any rack and pinion sub-stage, and can therefore be added without alteration to any microscope thus fitted.

Mechanical Stages.—These are a great convenience, and in some cases, such as measuring, systematic searching, blood counting, etc., are a necessity. The best forms unquestionably are those which are built into the stage, such as are supplied with most of the higher-class English stands. It is important that the bearings should be sprung, as any back-lash due to wear, or careless fitting, is a great annoyance. In this type of mechanical stage the opening should be wide, and, as already stated, the screws of the adjustment should not travel with the stage, otherwise one or the other will soon foul the top of the condenser.

A great many forms of detachable mechanical stage are made by various makers. They are convenient, but they are inferior to the non-removable type, as the best of them have a tendency to work loose sooner or later. On the other hand, they give a large range of movement. The types of these which are least satisfactory are those

which work by friction, as they are apt to become clogged or to wear unevenly, and those in which the milled heads are set vertically upright upon the stage. Fig. 59 represents a form made by various English makers, with minor modifications, whilst Fig. 60 represents the cheaper but less satisfactory form. Nearly all are provided with verniers reading to $\frac{1}{10}$ millimetre.

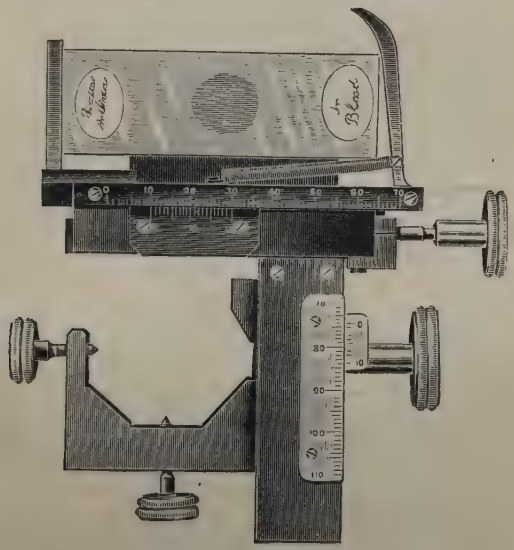


FIG. 59.—REMOVABLE MECHANICAL STAGE.

The convenience of the inexpensive sliding-bar already alluded to (p. 10) must not be overlooked. It makes an excellent substitute for the mechanical stage, giving a true vertical movement, whilst the slide is itself adjustable horizontally. When the stage is marked in small numbered squares of $\frac{1}{50}$ inch in the right or left hand corner, as in Fig. 14, known as Wright's 'Finder,' notes

of the position of an object on a slide can be recorded, as with the mechanical stages, or even rough measurements made.

Polarizing Apparatus.—**Polarizing Apparatus** is necessary for certain classes of work, mainly petrological and crystallographic, and gives beautiful effects on certain other objects, such as starch, textile fibres, hairs, horn

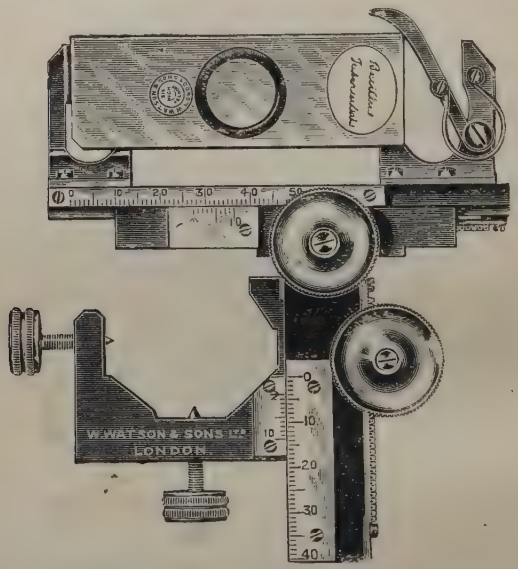


FIG. 60.—REMOVABLE MECHANICAL STAGE (CHEAPER FORM).

odontophores, etc. The **polarizer** (Fig. 61) fits into the sub-stage tube, where it is capable of rotation, and is generally graduated at intervals of 90 degrees or subdivisions of 10 degrees. The **analyser** (Fig. 62) either screws above the objective or is placed above the eyepiece. The latter place is preferable, as giving a brighter though smaller field. It also is graduated, and is capable

of rotation. In use the two Nicol prisms are adjusted so that their planes of polarization cross each other, in

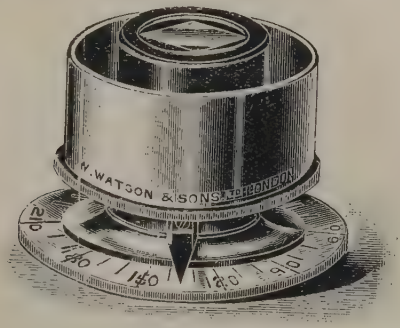


FIG. 61.—POLARIZER.

which case the ray of plane-polarized light emerging from the first prism, or polarizer, is entirely extinguished by

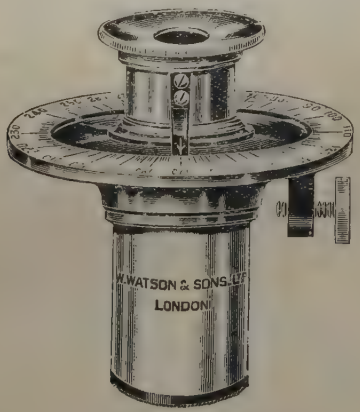


FIG. 62.—ANALYSER.

the second, or analyser, and complete darkness results. Any doubly refracting substance now placed upon the

microscope stage alters the refraction, and by its ' interference ' causes colour, or, at least, more or less marked variations of light and shade.

Selenites.—When the latter shades are very faint, due either to weak refraction or to extreme thinness in the object, it is advantageous to obtain a stronger contrast by using in addition a thin section of some more strongly refracting substance, such as **selenite** or **mica.** The selenites either fit in caps over the polarizer, or are mounted in separate rings, and sometimes are placed on the stage itself. The rotation of one of the Nicol prisms or of the selenite gives most beautiful effects.

It is, unfortunately, beyond the province of this book to go more deeply into the subject of polarization and its application by means of the microscope, and for this reason we must omit such accessories of the petrological microscope as the **quartz-wedge,** the **quarter undulation plate,** etc., and the **bi-quartz eyepiece,** nor must we deal with the means of showing rings and brushes in crystals by **convergent light.** It will, however, be found advantageous to have the polarizer so arranged that the optical part of whatever condenser is used fits also above the polarizer, and comes into focus with objects on the stage. This is of great service in militating against the serious loss of light due to the use of polarized light, especially with high-powered objectives and in photography.

Bull's-Eye Condenser.—The **Bull's-Eye Condenser** (Fig. 63) on the usual stand is a common adjunct of microscopes, but an unsatisfactory one, though Watson makes an aplanatic condenser, calculated by Mr. E. M. Nelson, which is especially useful in photo-micrography, as mentioned on p. 208. As ordinarily made, it is nearly hemispherical, and its chromatic and spherical aberrations are most marked. It can be used with transmitted

light to strengthen the illumination from a lamp, or for opaque objects requiring illumination on the stage (reflected light), where either artificial light or daylight

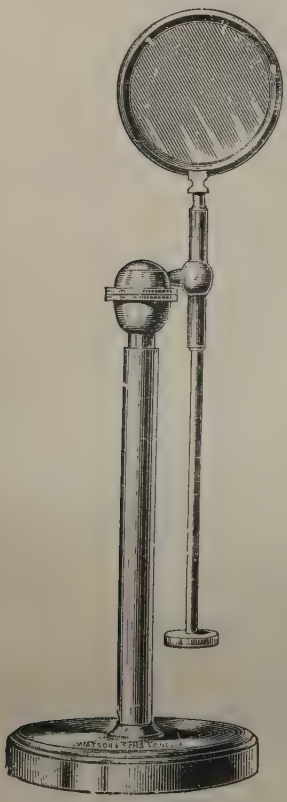


FIG. 63.—BULL'S-EYE CONDENSER.

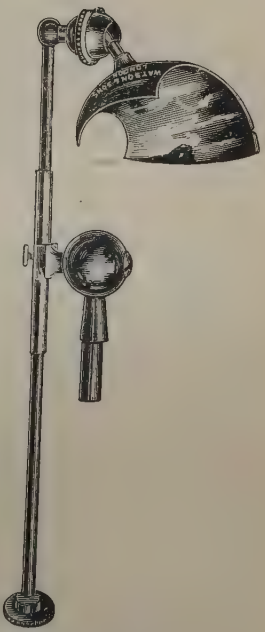


FIG. 64.—SIDE SILVER REFLECTOR.

is the source of illumination (see pp. 181 and 207). It is an advantage if this condenser is fitted with an iris diaphragm.

Side Silver Reflector.—For objects to be used by reflected light the **Side Silver Reflector** (Fig. 64) is most convenient and effective. The Lieberkühn is now little met with for reasons explained on p. 182.

If the tail-rod carrying the concave mirror will allow of the latter being adjusted above the stage—a little

FIG. 65.—VERTICAL ILLUMINATOR, WITH PRISM.

detail far too often omitted by makers—excellent illumination can be got by this means, as previously stated (p. 13).

Vertical Illuminator.—For illuminating opaque objects with high-power lenses, such as a $\frac{1}{12}$-inch immersion,

FIG. 66.—VERTICAL ILLUMINATOR, WITH COVER-GLASS REFLECTOR.

a **vertical illuminator** is necessary. This illuminator is screwed into the nosepiece of the microscope above the objective. The lamp is adjusted so as to throw a strong beam of light through the aperture at the side, and within is a prism (Fig. 65) or a cover-glass, or pref-

erably a thin optically worked glass disc, set at an angle of 45 degrees (Fig. 66), which reflects the light down through the objective on to the object, thus illuminating it. This piece of apparatus requires some care in adjusting, and can only be used for objects that are either uncovered or in actual contact with the cover-glass (see p. 182). Watson makes an improved form fitted with a small condensing lens, making the necessary adjustments easier, and with interchangeable prism and glass disc (Fig. 67). For critical work the objectives should be corrected for use without cover-glass. Of

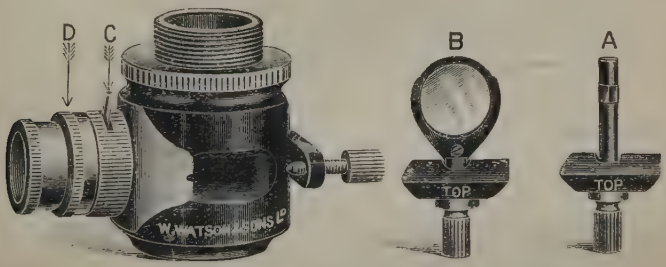


FIG. 67.—WATSON'S COMBINED VERTICAL ILLUMINATOR.

course, any movement of the tube in focussing alters the light adjustment, and some makers fit an illuminating lamp to this reflector (*cf.* Figs. 28 and 85).

Light Modifiers.—There are various means of modifying the illumination, of which the simplest is by means of a blue glass chimney or front glass to the lamp, or, better, by using discs of blue or yellow glass which drop into the ring beneath the condenser, or are held on separate stands. The blue glasses can be obtained in various depths of shade, but they are not truly monochromatic, as they pass some rays of other colours as well. For critical work, more especially in photo-

micrography, and with achromatic objectives, **Gifford's gelatine and glass screen** can be fitted into the same ring. It consists of a disc of green glass and a gelatine film, and gives approximately monochromatic light, though with considerable loss of light. It is, however, inexpensive. A better method, other than by the use of a costly prism arrangement, is **Gifford's fluid monochromatic light screen,** containing a slip of optically worked blue-green glass in a trough filled with a medium composed of aniline green and glycerine. This passes blue-green rays only, in the F portion of the spectrum, the red rays being completely absorbed, and does not reduce the illumination so much as the gelatine and glass screen.

Wratten's well-known light filters can be obtained 2 inches square, and mounted between optically worked plates. They are very efficient, and a complete set contains nine screens.

Recently Messrs. **Chance** of Birmingham, who have done so much for English optical and scientific glass, have brought out a set of glass filters for both visual and photo-micrographic work, covering wave-lengths from 4,300 to 7,000, and the selectivity of which is shown in the accompanying illustration (Fig. 68). They can be obtained from Watson and Sons, optically worked if required, 2 or 4 inches square, or $1\frac{3}{8}$ inch in diameter to fit in the ring beneath the condenser, and the price is very moderate.

The ordinary ' pot metal ' glass screens are much less efficient, and are not recommended.

All these screens are most useful in photo-micrography, where the actinic values of certain colours in stained preparations need to be rectified (see p. 219). The **ground-glass screen** often recommended may have its uses in giving a uniformly illuminated field, but it is

useless for critical illumination, whether placed beneath the sub-stage or immediately in front of the source of light.

Nosepieces.—A very useful accessory is a **nosepiece.** They are of two main types. The rotating nosepiece is the simplest and most convenient, but it has the drawback that it generally still further decentres the axes of the objectives from the axis of the microscope tube, frequently necessitating readjustment of the object with

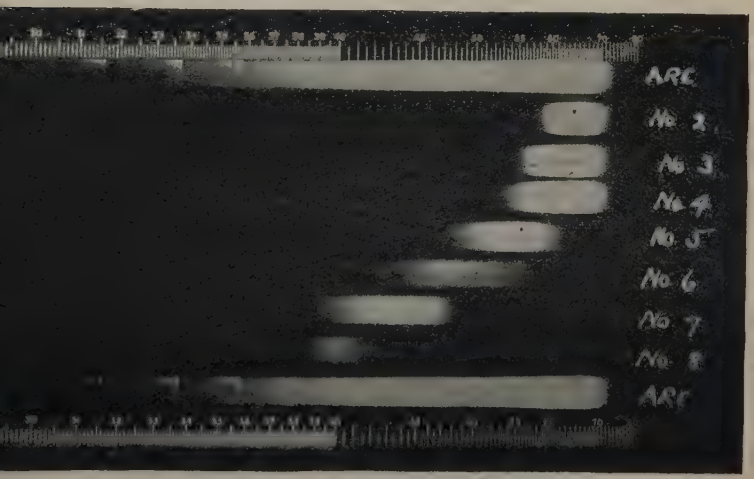


FIG. 68.—SPECTRUM OF CHANCE-WATSON FILTERS.

each rotation of a new objective into place, especially with high powers, and with critical illumination requiring re-centreing of the condenser. The rotating nosepiece are made to hold two, three, or even four objectives, and are now also made practically dust-tight. Four objectives, however, make a heavy weight for a fine adjustment to support, and the best form is the dustproof triple nosepiece (Fig. 69), made in aluminium.

The **objective changer,** as made by Zeiss, and other makers, has a tube-slide permanently attached to the microscope tube, and each objective is provided with its own corresponding slide, the latter containing adjust-

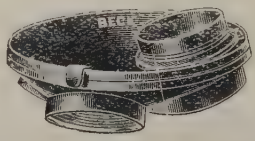


FIG. 69.—DUSTPROOF TRIPLE NOSEPIECE.

ments for centring, which is carried out once for all. Each objective is then slid into position as required, a case being provided to hold three or six objectives thus attached permanently to its own adjusted and centred slide.

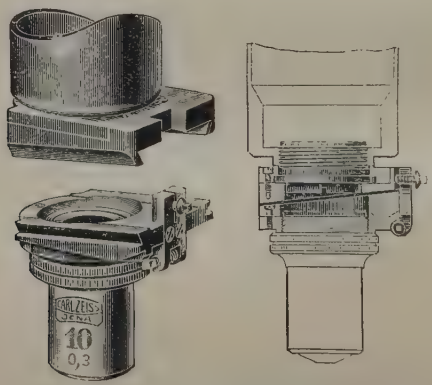


FIG. 70.—ZEISS'S OBJECTIVE CHANGER.

With all nosepieces it is, of course, a manifest advantage if the objectives are so mounted as to come roughly into focus without too much movement of the main tube.

It will be noted that they all add slightly to the tube-

length, and require proportionate closing in of the draw-tube.

Micrometric Apparatus.—Some means of measuring objects is sooner or later necessary to the microscopist. The simplest arrangement is that of a **stage micrometer** (Fig. 71), graduated in $\frac{1}{100}$ and $\frac{1}{1000}$ of an inch, or

FIG. 71.—STAGE MICROMETER.

$\frac{1}{10}$ and $\frac{1}{100}$ of a millimetre, whilst a small disc drops into the Huyghenian eyepiece, and lies just upon the diaphragm so as to be in the focus of the eye-lens. This latter is known as the **eyepiece micrometer** (Fig. 72), and is also ruled with divisions, generally bearing some

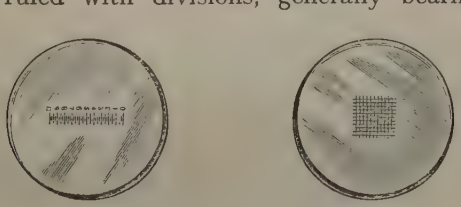


FIG 72.—EYEPIECE MICROMETER.

relation to an inch or millimetre scale. Sometimes the eyepiece micrometer is fitted to a plate running through the eyepiece, and adjustable by means of a screw for convenience in getting exact alignment. The most elaborate form is the **screw micrometer eyepiece** (Fig. 73), which is a beautifully accurate piece of workmanship,

by means of which, in skilled hands, the $\frac{1}{100000}$ of an inch can be spanned. It is a Huyghenian eyepiece, with

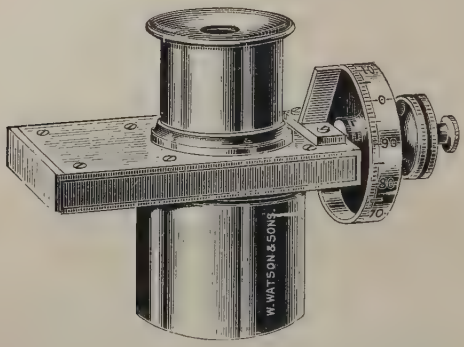


FIG. 73.—SCREW MICROMETER EYEPIECE.

a movable and a fixed wire set in the focus of the eye-lens (Fig. 74). The movable wire travels across the field

FIG. 74.—FIELD OF SCREW MICROMETER EYEPIECE.

by motion derived from a milled head and graduated drum. The latter is divided into $\frac{1}{100}$ parts. Within the eyepiece, in view of the eye-lens, but set to one side,

a sort of toothed comb is placed as a guide, one revolution of the external milled head causing the movable wire to travel from one tooth to another. Thus counting and measuring is facilitated.

Measuring.—To make measurements it is only necessary to note the number of arbitrary divisions in the eye-

FIG. 75.—ROUSSELET'S COMPRESSOR.

piece micrometer which correspond with the object to be measured, then to replace the object by the stage micrometer, and note the exact measurements which correspond to those taken in the eyepiece (see p. 193).

Compressor.—Stage Forceps are sometimes useful, and a **compressor** is often of service, such as Rousselet's form

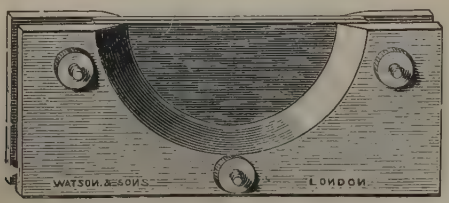


FIG. 76.—BOTTERELL'S TROUGH.

(Fig. 75), which is parallel, and easily cleaned, whilst broken glasses are readily replaced. The older form of 'live box' is messy, and less useful. A glass trough is a handy stage accessory, especially for pond-hunters, and perhaps the best form is Botterell's (Fig. 76), composed

of two slips of glass separated by an ordinary indiarubber ring, and held between two vulcanite plates, the whole being retained in position by three screws. The glasses can be easily cleaned or replaced.

Drawing Apparatus.—It is quite practicable to make good drawings direct from the microscope when used in the ordinary way, the paper being placed as near the stand as possible; but for accurate work some form of camera lucida is necessary.

Beale's Reflector.—The cheapest and perhaps the easiest to use is **Beale's neutral-tint reflector** (Fig. 77). It is simply a disc of tinted glass placed above the

FIG. 77.—BEALE'S NEUTRAL-TINT REFLECTOR.

eyepiece at an angle of 45 degrees to the optic axis. To use it, however, the microscope must be placed in the horizontal position, which is not always possible. The eye is placed above the glass disc, and looks down *through* it on the drawing-paper placed beneath. The microscopical image can then be traced. It is, however, transposed, though not inverted.

Wollaston's Camera Lucida.—Wollaston's Camera Lucida (Fig. 78) was very popular once, but is now little used. Like Beale's form, it necessitates the microscope being placed in the horizontal position. Its principle is that of a prism above the eye-lens reflecting the microscope image into the eye, but only half the pupil of the eye looks at this image, the other half looking down upon

the drawing-paper. The least variation in position of the eye makes one image much more distinct than the other, and altogether it is not an easy instrument to use.

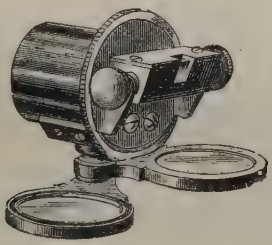


FIG. 78.—WOLLASTON'S CAMERA LUCIDA.

Combined Eyepiece and Camera Lucida.—A very useful and convenient eyepiece and camera lucida combined is sold by most opticians (Fig. 79). A prism reflects the

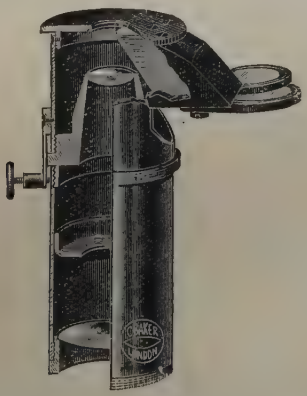


FIG. 79.—COMBINED EYEPIECE AND CAMERA LUCIDA.

drawing-paper and pencil into the eye through a second silvered prism, which has a central aperture through which the microscope image is viewed direct, and it

can be used with the microscope in either a vertical or inclined position. In the latter case, however, the paper must be inclined on a drawing-board, so as to be parallel to the object on the stage; otherwise there will be distortion of the drawing.

Abbe Camera Lucida.—The Abbe form is really an elaborated form of the preceding camera lucida, but it is the best and easiest to use (Fig. 80). It reflects the paper by means of a mirror of some size, which is attached

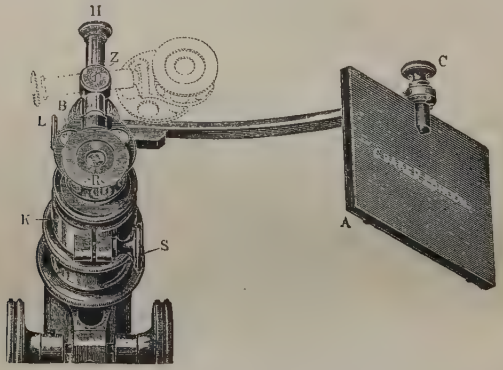


FIG. 80.—ABBE CAMERA LUCIDA.

to an arm projecting from a ring surrounding the eyepiece. Above the latter is a silvered prism again reflecting the image of the paper and pencil into the eye, the prism, as in the former type, having a small, clear space in its centre through which to view the microscope image. In its completest form the silvered prism above the eyepiece is provided with centring adjustments, and can be readily swung out of the way; and it is also provided with two sets of smoked discs of graduated densities to adjust the light, one set being between the prism and the eyepiece,

and the other set being between the prism and the mirror. By this means the difficulty of adjusting the respective brightness of the two images is largely overcome.

Any form of projection microscope can, of course, be used for drawing if the eyepiece is provided with a reflecting prism, and such a prism, or a reflecting mirror, can be used with an ordinary microscope in the horizontal position, but the light from the illuminant must be carefully shielded (see p. 71).

Lamps.—The lamp used by microscopists is generally of paraffin, with a $\frac{1}{2}$-inch wick, and it need not necessarily be other than the ordinary lamp, which can be bought anywhere for a shilling or two, and for which a cardboard screen with an aperture cut in it can easily be made at home. For all ordinary visual work, other than that requiring the use of deep screens, such a lamp, properly focussed, gives ample illumination, even with immersion lenses. The light is restful and grateful to the eye, where a stronger and whiter illumination is often absolutely injurious, and its flame edge, about $\frac{1}{2}$ inch high by $\frac{3}{16}$ inch wide, gives unequalled facilities for obtaining critical illumination.

The excellent and often elaborately fitted microscope lamps sold by opticians are, however, very useful. Their great convenience lies in the readiness with which they may be raised or lowered, and the flat receptacle for the oil which enables them to be brought close to the table. If a regular microscope lamp be purchased, it should certainly have this form, and the reservoir should be capable of rotation, so as to enable either the flat or the edge of the flame to be used. It should also have an iron chimney, holding an ordinary 3-inch by 1-inch glass slide, which can be easily exchanged if cracked. A reflector is worse than useless, as it confuses

the light rays. This type of lamp is shown in Fig. 81. Vertical and horizontal adjustments by rack and pinion are refinements only necessary for a limited class of work, requiring the utmost nicety of adjustment, but an attachment for an aplanatic bull's-eye of the Herschel or Nelson type is often of much service, especially in

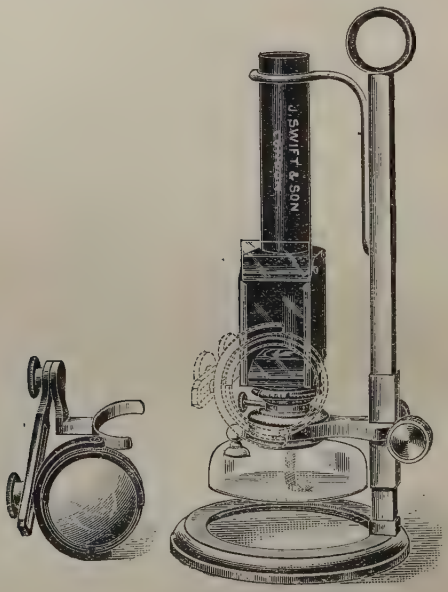


FIG. 81.—MICROSCOPE LAMP.

photography. Beck and Swift make most convenient lamps of this sort, with the bull's-eye swinging up and down, so that the flame always remains central. A flat receptacle for the oil, as already mentioned, is really essential, in order that the flame may come within 3 inches or so of the table, and this is, consequently, a primary requirement.

The **Heliostat,** which is a sunlight reflector working by clockwork to keep it in constant alignment, apart from its cost, has disadvantages too manifest in this country to be much used, though it has been arranged to give excellent and powerful monochromatic light with prisms or gratings.

The use and possible advantages of monochromatic illumination are dealt with on pp. 106, 188 and 219, and when this is required an oil-lamp, as already explained, is not sufficiently strong for use with deep blue or green screens such as Gifford's. A more powerful light is then necessary, but unfortunately an entirely satisfactory one has not yet been invented. The incandescent gas mantle is often used on the Continent, but if critical light is used the pattern of the mantle becomes unpleasantly obtrusive. The interposition of a ground-glass screen, sometimes recommended, is unsuitable, as it scatters the light, and prevents the use of critical illumination, with disadvantageous results, though it certainly gives an evenly illuminated field. The ordinary incandescent electric lamp, with its multiple filaments, is quite unsuitable for microscope illumination, but ordinary electric lamps with ground-glass bulbs, especially the Ediswan gas-filled 'Fullolite,' give a soft, diffuse light for uncritical work. The most satisfactory all-round electric illuminant yet discovered is the Nernst lamp (which is unfortunately no longer made, at present, at any rate), but its filaments when focussed give too fine bars of light even with immersion objectives and high-power condensers, and their life is also somewhat uncertain. A ground-glass disc is sometimes put in the centre of the bulb, but this, again, interferes with the obtaining of critical illumination. The 'Pointolite' lamp (Fig. 82) has a small ball of tungsten, which gives a small and intense

point of light, and can be obtained as low as 30 c.p., and as high as 4,000 c.p., for direct current, and of 150 c.p. (only) for alternating current. All these require a special resistance for starting and running. This is now the best electric lamp obtainable for microscope or projection work, but all electric lamps tend to give a light which is

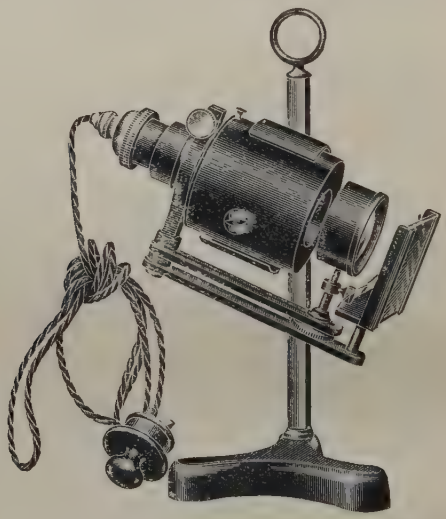


FIG. 82.—'POINTOLITE' ELECTRIC LAMP.

too strong for visual work, and therefore injurious to the eyes.

The arc light is much too powerful to be used other than in photo-micrography, though an excellent little **arc lamp**, with hand- or mechanical-feed, using only 4 ampères, is sold by various makers, and is useful for this purpose and for high-power, dark-ground illumination (Fig. 83). The carbons are set at right angles, and the lamp can be used with either direct or alternating

current at a voltage of about 60. In the case of a higher supply from the mains a suitable resistance must be interposed. The writer uses a large jam-pot filled with salt solution, two lead plates forming the terminals, and the resistance being adjustable by moving the plates round the rim of the jam-pot! The drawback of the arc lamp for photography is its liability to decentre the light at critical junctures.

Mr. J. E. Barnard has given particular attention to the **Mercury Vapour Lamp,** in which an electric current

FIG. 83.—SMALL ARC LAMP.

is passed through a quartz tube containing mercury. This renders the mercury vapour luminous, and gives light that is confined to a few wave-lengths in the ordinary spectrum, which are widely separated, and to certain ultra-violet rays, and thus readily permits of the use of monochromatic light, by means of suitable screens, in several regions of the spectrum, and so affords good contrast with different stains. The mercury vapour lamp can also be used for photography only with certain ultra-violet radiations, through a quartz condenser, thus obtaining the advantage of short wave-lengths (see p. 164)

The 'K.B.B.' mercury vapour lamp (Fig. 84) has a fused quartz envelope giving ultra-violet radiation, and has the additional advantage of not being easily damaged.

Various metal casings are obtainable for enclosing electric bulbs of the ordinary or ground-glass type. They should all be capable of adjustment.

Certain makers supply small lamps as permanent attachment to a dark-ground illuminator or to a vertical

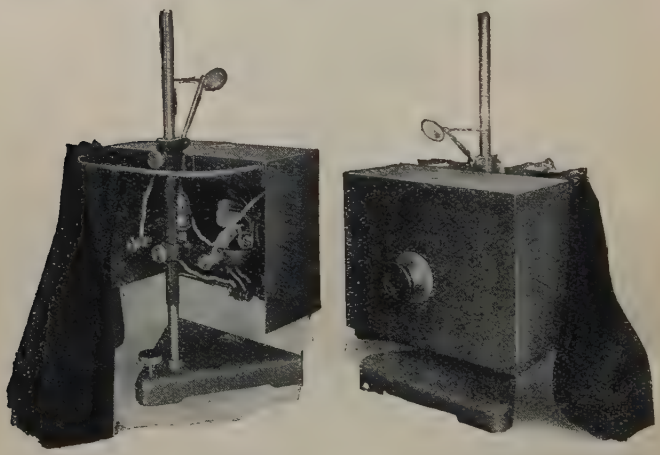


FIG. 84.—'K.B.B.' MERCURY VAPOUR LAMP.

illuminator, thus saving troublesome adjustment and ensuring the most favourable results (Fig. 85; *cf.* Fig. 28).

For photo-micrographic work the oil-lamp; small, single, acetylene jet; Nernst or 'Pointolite' electric lamp; oxy-hydrogen burner; or arc lamp may be used, of which the last is the best, when it can be obtained. The oxy-hydrogen burner is useful where electric light is not available, and is very convenient, but the acetylene

jet has good actinic properties, and deserves to be more widely known in this connection.

Davis Shutter.—A '**Davis Shutter**' is merely an iris diaphragm mounted in a ring so as to screw above the objective. It is useful in photography as a means of cutting off flare at the sides of the tube, and, as it reduces the aperture of the objective, it is also sometimes of service in increasing the 'penetration' or depth of focus, though, of course, at the expense of the definition. If a carrier to fit into the sub-stage is provided, the Davis shutter, being fitted with the Society's screw, enables a

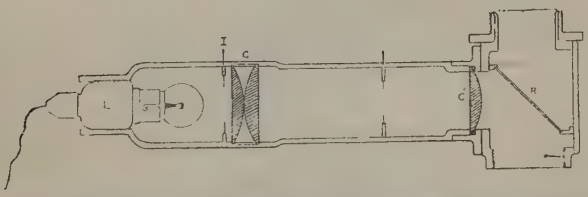


FIG. 85.—WATSON'S COMBINED LAMP, CONDENSER, AND VERTICAL ILLUMINATOR.

low-power objective to be used as a condenser, and provides an efficient iris diaphragm.

Ehrlich Eyepiece.—This is an eyepiece made with an iris diaphragm, giving a square opening which can be varied in size. It is useful in making blood counts.

Indicator Eyepiece.—This is an ordinary eyepiece provided with a pointer, movable from outside, which, combined with rotation of the eyepiece, will indicate the position of any particular portion of a slide. Crossed cobwebs are used for the same purpose.

Object Marker.—A means of permanently indicating any particular portion of a slide is often of service, and Leitz makes an apparatus suitable for the purpose, which

screws into the nosepiece after removal of the objective, and has a writing diamond, which, on rotation, cuts a small circle in the cover-glass (Fig. 86). It is provided with a spring to give the necessary pressure, and an adjustment for size of circle. This instrument is also made by Beck.

Nosepiece Shutter.—This is an attachment made by Beck, screwing above the objective, and containing a sliding-plate for making exposures in photo-micrography. It is useful where a simple camera of short focus, un-

FIG. 86.—LEITZ'S OBJECT-MARKER.

provided with a shutter, is being used, but it naturally tends to shake the microscope, and thus to disturb the focussing.

Hot Stages.—These are made by most makers, and are variously heated by the direct action of heat from a lamp to a metal projection, by circulating hot water, or even by electricity. Baker makes a growing cell, designed by Mr. Merlin, with water reservoir and circulation system, by means of which an organism can be kept under observation for weeks without fear of evaporation.

Micro-Spectroscope.—The adaptation of the spectroscope to the microscope has hitherto not been found of

much service owing to the delicacy and greater convenience of spectroscopic examinations by other methods, and will therefore only be mentioned here. The instrument, as made by Zeiss, is provided with an adjustable slit, a comparison prism with stage and mirror, and a wave-length scale with mirror.

FIG. 87.—LEITZ'S MICROMETER GAUGE.

Cover-Glass Gauges.—These are useful when much high-power work is being done, necessitating attention to the thickness of cover-glass and slide used, especially with dark-ground condensers (see p. 95), to avoid interference with the corrections and performance of the objectives or condensers (see pp. 135 and 185). Suitable forms are made by various makers. Beck's is of the lever type, but the others are micrometer screws. The illustration

shows the micrometer gauge type as made by Leitz (Fig. 87).

Troughs.—These are convenient for holding solutions for monochromatic light, for absorbing heat rays in photo-micrography and projection, and for other purposes, such as the study of pond-life. Kingsford's troughs, as made by Watson, obviate the tendency to leak, which is a common fault of such troughs, and consist of an outer brass strap lined with rubber, which holds together the glass discs of the sides. They are made in various sizes, either to interchange with the bull's-eye, or

FIG. 88.—EYE-SHADE.

on independent stands. Beck makes a very neat stand for monochromatic light, and a double wedge light moderator on an adjustable stand, which contains two neutral tinted glass wedges sliding in opposite directions to vary the thickness of neutral glass, and which can also take colour filters or diaphragms.

Eye-Shade.—Workers who have a difficulty in keeping both eyes open when looking down a microscope tube (a most necessary proceeding) may find the inexpensive attachment illustrated (Fig. 88) useful. It is supplied by most English makers, and the ring fits over the eye-piece end of the tube.

CHAPTER VI

THE PRACTICAL OPTICS OF THE MICROSCOPE

It is unnecessary to give here a detailed explanation of the principles underlying the use of lenses, and the formation of real and virtual images. Under any circumstances the treatment would necessarily be inadequate within the limits of space at our disposal, but the beginner will find it to his advantage to consult some elementary work on physical optics, or one of the larger books dealing with the microscope, in order to obtain a clear idea of the subject. For the understanding, however, of many matters connected with the microscope, it is necessary to bear in mind the cardinal fact that a lens is in reality but an assemblage of superposed prisms, and that light in passing through a lens is accordingly *refracted*, *deviated*, and *dispersed*.

Refraction.—The **Angle of Refraction** is dependent upon the fact that a ray of light passing into a denser medium is refracted or bent *towards* the perpendicular, and that a similar ray of light passing into a rarer medium is, conversely, bent *from* the perpendicular. This is illustrated in Fig. 89, where A is the incident ray in air which, entering the block of glass represented by the shaded lines, is refracted *towards* the perpendicular or ' normal ' D, as represented at B, and, emerging again into a rarer medium, is again refracted *from* the perpendicular at C. In A and B we have refraction, and in A and C lateral displacement due to refraction. The amount of refraction will vary according to the

respective densities of the media, but will remain constant in the relation any two media bear to each other, and the *ratio* of refraction in the same medium will be the same, whatever may be the angle of incidence. This will be made clear by the accompanying diagram (Fig. 90), where we have a dense medium, which we will suppose to be glass, through which a ray of light, A, is passing, and coming out at A′. We must note several things about this ray. Let N be a perpendicular to the surface of this medium, known as the ' normal.' Then we shall observe that on emergence to a less dense medium, such as air, A

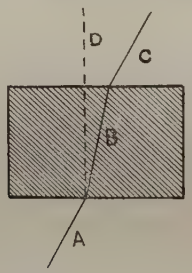


FIG. 89.—REFRACTION OF LIGHT.

becomes refracted *away from* the normal towards A′; and if we measure the distance from A to the normal, at right angles to the latter, in the glass medium, we shall find it bears a definite ratio to a similar line drawn from A′ outside the glass. Trigonometrically, these lines are, of course, known as the sines of the angles made by these rays with the normal. Now, if A be shifted farther away, say to B, it will be found that B, on emergence, becomes still farther refracted away towards B′, but on measuring the sines of these angles it will be found that they bear exactly the same *ratio* to each other. This is Snell's law, an important one

in microscopical optics, which states that in one and the same medium the sine of the angle of incidence always bears a definite ratio to the sine of the angle of refraction. Therefore, if we know the angle of incidence and the angle of refraction, we can easily find the refractive index, or *vice versâ*.

Let us now suppose A to be shifted still farther away to C. Here it will be found that the ray is now unable to emerge at all, as C′ corresponds with the surface of the

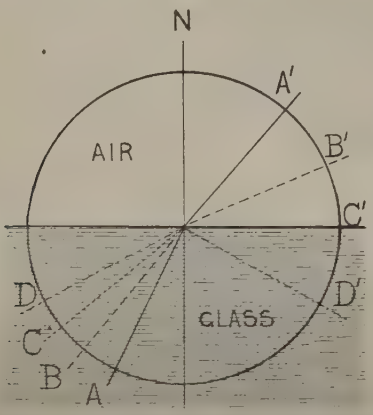


FIG. 90.—DIAGRAM ILLUSTRATING LAW OF REFRACTION.

glass medium, and therefore we shall have arrived at the 'critical angle,' where the refracted ray cannot emerge from the denser medium to which its refraction is due. Carried farther, say to D, the ray will be found to be totally reflected back at the same angle, as at D′. This is readily demonstrated by holding a glass of water above the eye, so that the surface, looked at from below, appears to be of a mirror-like brightness. The critical angle of water, measured from the perpendicular, is about $48\frac{1}{2}$ degrees, and of glass about $40\frac{1}{2}$ degrees.

Refractive Index.—Taking air, therefore, as unity, it is easy to calculate the amount of refraction all other substances bear to air, and, by dividing the sine of the angle that the incident ray makes with the perpendicular by the sine of the angle that the refracted ray makes with the perpendicular, to arrive at the *refractive index*. Thus calculated, the refractive index of water may be taken as 1·334, of cedar oil as 1·510, of Canada balsam as 1·526, of fluor-spar as 1·4338, of crown glass as 1·51 to 1·56, of flint glass as 1·54 to 1·71, and of realgar as 2·549.*

Deviation.—The **Angle of Deviation** is dependent upon the fact that an incident ray entering a prism is, in

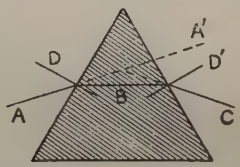


FIG. 91.—REFRACTION OF LIGHT THROUGH A PRISM.

accordance with the foregoing law, refracted towards the perpendicular, but the sides of the prism being inclined towards each other, the perpendiculars vary for the two sides, and the ray on emergence is therefore again refracted, and in consequence deviated still farther in the same direction. Fig. 91 shows this clearly, where AA′ is the incident ray, which is refracted at B towards the perpendicular D, but emerges at C refracted away from the new perpendicular D′.

It is scarcely necessary to add that upon the amount of deviation of the rays of light by a lens depends the focal length and consequent magnifying power of the latter.

* Carpenter, 8th edit., pp. 1108, 1109.

Dispersion.—The **Dispersion** is dependent upon the fact that, as white light is composed of rays of different wave-lengths and of different colours, the shorter waves are more refracted than the longer waves in passing through a prism, and in consequence the resulting ray of light will be fanned out into a *spectrum*, of which the violet rays will be the most and the red rays the least refracted. This is purposely not represented in Fig. 91.

The practical application of the foregoing explanations will be evident on referring to Figs. 92 and 93, which represent the passage of rays of light through a double convex and a double concave lens respectively. In

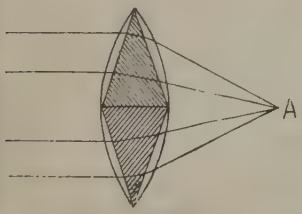


FIG. 92.—REFRACTION OF LIGHT IN DOUBLE CONVEX LENS.

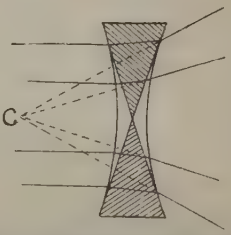


FIG. 93.—REFRACTION OF LIGHT IN DOUBLE CONCAVE LENS.

the convex lens (Fig. 92) parallel rays of light fall upon the surface of the lens, whose curvature gives varying normals, with the result that the rays converge to a 'real' focus at A. In the concave lens (Fig. 93) the opposite happens, and the rays diverge as if they converged upon a point, C, on the *anterior* side of the lens, called the 'virtual' focus. In the convex lens the focus A, formed by the parallel rays of light, is spoken of as the 'principal focus' or 'focal point,' and in a simple lens of this description the 'focal length' will be the distance from A to the centre of the lens. If, on the other hand, the radiant were placed at A, a parallel beam

of light would be given off from the other side of the lens. If the radiant were placed nearer to the lens than A, a divergent beam would be given off, whilst if it were placed beyond A, a converging beam would result, coming to a focus, which is spoken of as a 'conjugate focus.' Fig. 94 illustrates various types of lenses.

Magnification.—The magnifying power, or the converse, depends upon the spherical curves of the lens or their distance from the centres of curvature—*i.e.*, the 'radii'—combined with the direction of the rays of light. Primarily, a double convex lens, with a radius of 1 inch,

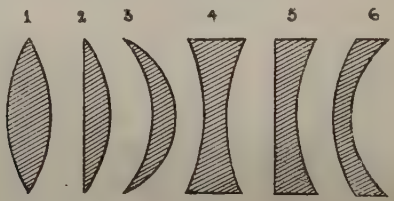


FIG. 94.—TYPES OF LENSES.

1, Bi-convex; 2, Plano-convex; 3, Converging meniscus; 4, Bi-concave; 5, Plano-concave; 6, Diverging meniscus.

will have a focus of 1 inch, whilst a plano-convex lens will, of course, have a focus of twice that amount—*i.e.*, 2 inches. The effect of the convergence can be seen by reference to Fig. 95, where conjugate foci are set up, with the result that the rays from the image A B, are converged by the lens C so as to enter the eye as parallel* or slightly divergent rays, this being necessary for focussing upon the retina. The image is normally *seen* at a distance of about 10 inches from the eye, the rays forming a virtual image at *a b*. We will speak later of the determination of the magnifying power of a lens,

* Not parallel with the optic axis of the microscope.

but in the meantime it is necessary to note that the actual magnification at *a b* with any specific lens will depend with each observer upon whether his eye is able to focus the object so as to give a virtual image at a distance of about 10 inches from the eye. If the eye be short-sighted, and the image be formed at, say, 7 inches, it will be apparent that the magnification through a lens will be less than that obtained by the normal eye, whilst the converse applies to the long-sighted observer. The ratios are not, however, in the

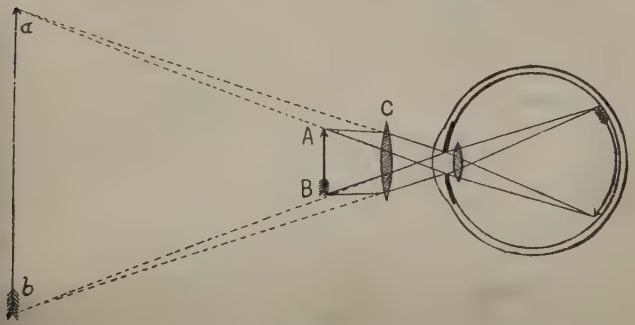


FIG. 95.—MAGNIFICATION, AND FORMATION OF A 'VIRTUAL' IMAGE.

proportion of 7 to 10, as the determining factor is the angle entering the eye and the consequent projected image, and, as a matter of fact, the difference in magnification through the lens is not great.

Aberrations.—There are two important defects inherent in a simple lens, and, indeed, to a less extent in all compound systems of lenses hitherto constructed. These are known respectively as **spherical** and **chromatic aberration.** The rays of light passing through the margin of a lens, however carefully made, do not, unfortunately, come to a focus in exactly the same place as

the rays passing through the central part. The double convex lens is one of the worst offenders in this respect, whilst the plano-convex lens is generally less imperfect, provided the most favourable position is selected. This will be understood by reference to Figs. 96 and 97. In Fig. 96 the marginal rays come to a focus nearer the lens than the more central rays, a state of affairs known as 'under-correction,' whilst in Fig. 97 the central rays come to a focus nearer the lens than the marginal rays, which is known as 'over-correction.' In the cheapest lenses this is made less evident by shutting off the marginal rays by means of a diaphragm, with consequent

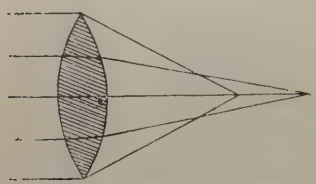


FIG. 96.—SPHERICAL ABERRATION (UNDER-CORRECTION).

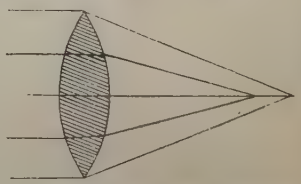


FIG. 97.—SPHERICAL ABERRATION (OVER-CORRECTION).

loss of light and reduction of aperture, upon which 'resolving power' so largely depends. In higher-class lenses the spherical aberration is reduced by combining lenses of varying curvatures which are composed of glasses of different densities, the eyepiece also doing its share of the corrections, especially in the apochromatic systems. The correction of the spherical aberration is known as **aplanatism.**—In addition to this, the lens *disperses* the light, as explained on p. 129, the amount of dispersion varying according to the refracting angle of the lens and the refractive power of the glass of which it is composed, so that the violet rays at one end and the red rays at the other end of the spectrum

are brought to a focus independently of each other at different distances from the lens. This is known as **chromatic aberration.** Figs. 98 and 99 represent a convex and a concave lens respectively, the dotted line representing the violet or most refracted ray, and the black line the red or least refracted ray. One prism can

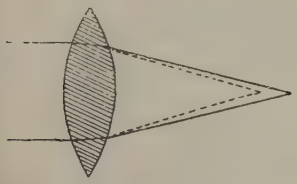


FIG. 98.—CHROMATIC ABERRATION (CONVEX LENS).

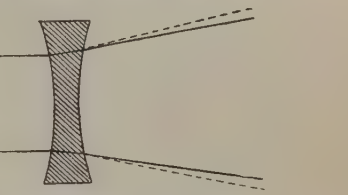


FIG. 99.—CHROMATIC ABERRATION (CONCAVE LENS).

be made to correct another, as in Fig. 100, but only by destroying the refraction, and the remedy is, therefore, to combine two lenses made of glass of different refraction, but corrective dispersion—for instance, a crown and a flint glass, as represented in Fig. 101—so as to

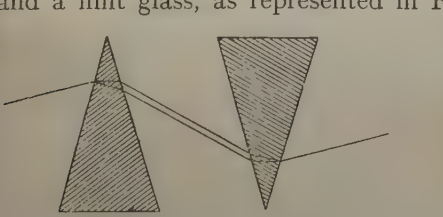


FIG. 100.—CORRECTION OF DISPERSION BY PRISMS.

FIG. 101.—ACHROMATIC COMBINATION.

neutralize the dispersion without destroying the refraction. This is known as **achromatism.**

Irrationality of Spectrum.—Unfortunately, there is a further difficulty to be overcome owing to the fact that the ratio of dispersion in a prism or lens is not the same for all, or indeed any, of the colours of the resulting

spectrum, and that no two glasses of different refractive indices, but proportional dispersion, can be obtained. This is known as the 'irrationality of the spectrum.' It will be seen that the optician has, therefore, no easy task in endeavouring to correct both spherical and chromatic aberration, and the colour left outstanding in an achromatic combination in which two colours have been successfully combined is known as the 'secondary spectrum,' a not too happy denomination. It may be added that the colours thus in practice combined are those which have the greatest light intensity, the spherical aberrations being corrected for the yellow-green portion of the spectrum, whilst the chromatic aberrations are corrected for the orange-yellow and green-blue portions. There are other corrections to be made by the computer, such as for 'coma,' due to non-fulfilment of the sine law, 'zonal aberrations,' etc., but these are mainly of technical interest, and outside the scope of this book. A more practical point for the worker is that a semi-apochromatic objective will always show some slight colour when a difficult object, such as a diatom, is looked at, and that this colour should be of a faint apple-green, this being the selected colour for correction, because it is that which is brightest for visual observation.

Jena Glass.—These corrections have been greatly facilitated by the researches of Professor Abbe, in conjunction with Dr. Schott, and the resources of the well-known glass-works of Schott and Co., and the optical firm of Carl Zeiss, both of Jena. For this purpose the German Government made a substantial grant of money, and the result has more than justified the expenditure, providing a notable object-lesson to other countries. Professor Abbe and his *confrères* succeeded in producing

new forms of optical glass, and in constructing new lenses, which have become famous throughout the world, and the prestige thus given to the firm of Carl Zeiss has given them an enviable position in the manufacture, not only of these lenses, but in optical apparatus of all kinds. The 'Schott' or 'Jena' glasses (now made in this country) are now used by all leading opticians both in this country and abroad, and the further improvement in achromatic lenses is sufficiently marked to justify their description as 'semi-apochromatic,' for they closely rival the 'apochromatic' lenses. The respective merits and advantages of these lenses have been already dealt with, but we may explain here that in the apochromatic objectives Professor Abbe, by means of original formulæ and the combination of new glasses of varying refractive and dispersive properties (notably those containing fluorite, which has a comparatively low refractive index and very little dispersion), succeeded in almost entirely eliminating the secondary spectrum, and thus uniting *three* different colours of the spectrum in one point of the axis, whilst at the same time correcting the spherical aberration for two different colours, and therefore practically for all, in contra-distinction to the usual correction for one colour in the brightest portion of the spectrum only.

It is to be noted that in the apochromatic the ocular takes part in the necessary corrections, and is *over-corrected*, so as to have a compensatory chromatic difference of magnification, the image formed by the red rays being larger than that formed by the blue rays—the exact opposite to that in the *under-corrected* objectives.

Disturbance of Corrections by Cover-Glass.—The thickness of the cover-glass over an object has an important influence upon the corrections of an objective. In Fig. 102 the rays of light emanating from an object at

A are refracted towards the perpendicular on entering the denser medium of the cover-glass B, according to their angle of incidence, and on emergence their direction is so altered that a condition of under-correction, as in Fig. 96, results, this being shown in Fig. 102 by the dotted lines. The result is that over-correction is set up in the objective. To remedy this, the objective, when used under such circumstances, must itself be under-corrected. It will be apparent, therefore, that the corrections of an objective will be altered not only for uncovered, as compared with covered, objects, but also for cover-glasses of varying thicknesses. A thicker

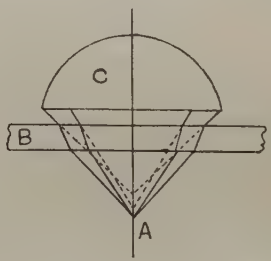


FIG. 102.—INFLUENCE OF COVER-GLASS.

cover, for instance, requires to be met by a corresponding amount of increased under-correction in the objective, whilst a thinner cover requires over-correction. This necessary under-correction of the objective is obtained by bringing the lenses closer together, and high-power 'dry' objectives of the best quality are generally provided with a rotating collar, which enables the distance between the combinations of the objectives to be adjusted for variations of cover-glass.* A very general method is to

* The correction collar should not move the front lens, a cheap but unsatisfactory method of construction, and one liable to damage objective or slide.

shorten or lengthen the microscope tube, which also rectifies such variations within certain limits. Thus, supposing the objective to be corrected for a cover-glass 0·008 inch thick, it would be necessary to shorten the tube—*i.e.*, decrease the distance between eyepiece and objective—if a thicker glass were used, or to lengthen it for a thinner glass. By so doing we should, of course, also alter the magnification. With a correction collar we should close the lenses of the objective for a thicker cover, and open them for a thinner one.

The correction of objectives by either of the above means requires considerable practice if the best results are to be obtained—results which the eye sometimes needs educating to perceive.

It would be a great convenience if makers would adopt one uniform thickness of cover-glass to which all objectives could be corrected, or if the Royal Microscopical Society could standardize a definite thickness. One well-known maker corrects for a cover-glass 0·006 inch thick, whilst another corrects for a 0·008-inch cover-glass.

Objectives are likewise corrected for a certain definite tube-length, and it will scarcely be necessary to point out, after what we have previously said, that an objective corrected for a certain length of tube cannot be used *critically* for any other length of tube, except as mentioned above. This, whilst showing the advantage of having a draw-tube, shows also the limitations of variation of magnification by the simple device (sometimes suggested) of drawing out or closing up the draw-tube. The standard English tube-length was formerly 10 inches, but the now almost universal use of the short Continental tube-length has caused English opticians also to correct their objectives for this tube-length. We regret this for

many reasons, one of which is that an objective corrected for the 10-inch tube performs better on a 6-inch tube, if necessary, than in the reverse case, and also for the reason that we believe it is better to obtain a given magnification by using a 10-inch tube, and keeping the power of the eyepiece as low as possible (see p. 157). But we again protest against the practice of certain makers of quoting their objectives as giving certain magnifications calculated for a 10-inch tube, but refraining from adding the necessary qualification that the said objectives are in reality corrected and intended to be used for the 6-inch tube only. It would be an advantage if makers would mark upon their objectives, as some firms already do, not only the N.A. and the actual focal length of the objectives, but also the tube-length for which they are corrected. The Continental system of designating objectives and eyepieces by arbitrary letters or numbers has nothing in its favour.

Dry and Immersion Lenses.—It will be seen that the dry lens used in air is subject to many disturbing influences which react unfavourably upon its performance, and this is especially noticeable with high powers, such as $\frac{1}{8}$-inch, particularly when used with high-power oculars. Owing to the refraction of the rays when entering the air-space between the cover-glass and the objective, a considerable proportion of them fail to enter the latter, and accordingly objectives have been specially constructed and corrected for use when immersed in a medium placed between the objective and the cover-glass. It will be manifest that for contact to be maintained such objectives must have a comparatively short working distance, and **immersion lenses,** as they are called, are therefore always high-power lenses, generally $\frac{1}{8}$-inch or $\frac{1}{12}$-inch. The medium may be water, oil, or, in the case

of one exceptional and costly lens (see p. 77), monobromide of naphthalene. Whilst a water-immersion lens gives much better results than a dry lens, it is inferior in performance to an oil-immersion lens. The oil universally used is thick cedar-wood oil, which has a refractive index practically the same as crown glass—*i.e.*, 1·52—so that the cover-glass becomes to all intents and purposes part of the objective. This is accordingly spoken of as a **homogeneous immersion lens.** The refractive index of water is 1·334, air being taken as 1·0. With a homogeneous lens there is little or no disturbance due to variation in thickness of cover-glass, and no need, accordingly, for a correction collar. But the most important point is that, owing to the fact that none of the rays of light emanating from the object are subject to diffraction and are thus bent out of their course, an oil-immersion lens utilizes a larger number of rays than a water-immersion, and a much larger number of rays than a dry lens. Moreover, these rays are those which have most value, the intensity of the emitted rays becoming less as they form a larger angle with the perpendicular. As a matter of fact, an oil-immersion lens with an angular aperture of 82 degrees receives an equal number of rays to a water-immersion with an angle of 97 degrees, or a dry lens with an angle of 180 degrees, could so large an angle be obtained. Water-immersion lenses are not often used now except for objects that would be harmed by oil.

Angular Aperture.—The **Angular Aperture** of a lens is the extreme angle of the rays diverging from any point in the object which can enter the objective. The mere *diameter* of the front or back lens of an objective is not understood by the term ' aperture '—in fact, it generally happens that the ' aperture ' is in an inverse proportion

to the diameters of various lenses. But, owing to the introduction of immersion lenses, it is apparent that the mere statement of angular aperture does not express the relation that different lenses, say a dry and an immersion lens, bear to each other. Professor Abbe, to whom modern microscopy owes so much, introduced in 1873 a formula to which all systems can be referred for comparison, and which represents the ratio between the radius of the *effective* aperture on the side where the image is formed—*i.e.*, at the back instead of the front of the objective—and the equivalent focal length of the objective. This resolves itself mathematically into the sine of half the angle of aperture multiplied by the refractive index of the medium between the front of the objective and the cover-glass. The formula is, therefore, n sine u=N.A.,—n being the refractive index, sine u the sine of half the angle of aperture measured at the back of the lens, and N.A. the **numerical aperture.** This last term has now come into general use. The *Journal of the Royal Microscopical Society* contains a table in which the various values have been calculated out, and to which reference may be made (see Appendix, p. 325, Aperture Table).

Rating of Objectives.—It may be as well to repeat here that the terms ' 1 inch,' ' $\frac{1}{4}$ inch,' etc., do not represent the distance between the under side of the lens and the object. The tendency of the day is to increase the aperture, bringing the lenses closer and closer to the object, and the designations referred to may be taken practically as representing approximate magnifying power. A combination of lenses making up an objective of 1-inch power is treated as having an *equivalent focus* to an objective containing only a single lens with a radius of 1 inch on both sides and a consequent focus

of 1 inch. Such a lens, when focussed upon an object, will give a real image upon a cardboard screen placed 10 inches from its upper principal focal plane of ten times the actual size of the object. Ten inches is assumed to be the normal visual distance, and taking the inch, therefore, as a standard, we may say that

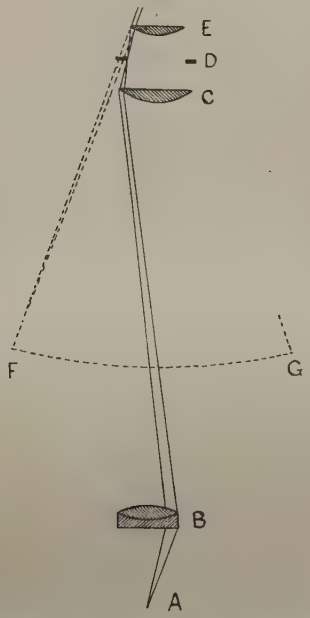


FIG. 103.—DIRECTION OF LIGHT IN A COMPOUND MICROSCOPE.

without any eyepiece it gives in a 10-inch tube a magnifying power of approximately 10 diameters. Put in another way, let us say that if we hold an object 10 inches away from the eye (which is about the usual distance for clear vision), it will look ten times as big when magnified by an inch objective without eyepiece. A $\frac{1}{4}$-inch gives

40 diameters, a $\frac{1}{6}$-inch 60 diameters, and so on. To get the actual magnification of a microscope, we must also know not only the length of the tube, but the magnifying power of the eyepiece in addition. Fig. 103 shows the path of the rays in a compound microscope. The rays of light from the object A, upon which the objective is focussed, pass through the objective B, and, crossing the rays from the other side, would, if uninterfered with, form a real image beyond the position of the diaphragm D. But the eyepiece is so constructed that the field-lens C intercepts the rays shortly before they reach the position necessary to form such a real image, and bends them in, forming the real image in the air in the position defined by the diaphragm D. This image is again magnified by the eye-lens E, a magnified virtual image being projected at F G in the same way as explained on p. 131, by means of Fig. 95.

It will save misunderstanding if we say here that the word 'diameters' means linear magnification, and not areas. Ten diameters means ten times as long, or ten times as wide, and not $10 \times 10 = 100$ times as big!

Effect of Tube-Length on Magnification.—It is evident from what has been said that the initial magnification of an objective will vary according to the distance of D from the objective, this being the position where the real image is focussed; and if the microscope tube be shortened or lengthened, not only will re-focussing be found necessary, but the magnification will vary proportionately. If the eyepiece magnifies six times, then an inch objective with this eyepiece and a 10-inch tube will give a total magnification of 60 diameters, a $\frac{1}{4}$-inch will magnify 240 diameters, and so on. With a 6-inch tube the magnification will be only six-tenths of the foregoing, and this must carefully be borne in mind.

We may repeat the necessary qualification that the frequently met with instructions as to varying the magnification by altering the tube-length are not practicable for critical work with objectives of high or even moderate aperture; an objective must be used with the tube-length for which it has been corrected, due allowance being made for thickness of cover-glass as before explained.

Opticians have, unfortunately, as previously stated, a tendency to give their objectives rather larger magnifications than those we have mentioned, partly owing to the difficulty of making low-power objectives of as wide apertures as the taste of the day demands, and this should be noted when purchasing. It is easier to make high-power objectives of a given large aperture than low-power ones. The higher the aperture, the better should be the definition, but the higher also is the price, and there is the additional disadvantage to many workers that anything out of the exact plane in focus is practically invisible. Most workers use objectives of both high and low aperture, and each has its advantages.

Magnification of Objectives—Initial Magnification.—The easiest way to test this is by the method mentioned by Carpenter. A micrometer slide, ruled in hundredths and thousandths of an inch, or in tenths and hundredths of a millimetre, is placed upon the stage of the microscope, and the latter inclined to the horizontal position. A strong light is transmitted through the microscope, and the room darkened. The micrometer lines are then focussed sharply upon a piece of white cardboard placed 5 feet (60 inches) behind the front lens of the objective. The divisions on the screen are measured with an ordinary foot or millimetre rule, and the result divided by six, which gives, of course, their size at 10 inches from the objective. The value of the original

stage micrometer divisions being known definitely beforehand, it is easy to calculate the resulting magnification. Suppose the distance between micrometer rulings of two $\frac{1}{100}$ parts of an inch to measure $1\frac{1}{4}$ inch at 5 feet distance, with a nominal 1-inch objective, then at 10 inches distance they would measure 0·2083 inch, which is equivalent to an initial magnification of nearly ten and a half times. A millimetre scale or rule can be used on the basis of 25·4 millimetres to the inch. We repeat that magnifications are always expressed in diameters or *linear* measurements, not in areas. A considerable distance such as the above is taken in order to reduce the amount of error due to the fact that the measurements should really be taken from the principal posterior focus of the objective, which in a compound system cannot easily be found. But by measuring from the front lens as above a very small margin of error is left. It is best to take the mean of several micrometer divisions, as they are not quite accurately ruled.

Combined Magnification.—Combined Magnification of eyepiece and objective is calculated by a similar method, except that there is not the same necessity for taking a longer distance, and the image of the micrometer must be accurately projected exactly 10 inches from the eye-lens of the eyepiece. This may be done either direct by means of a photo-micrographic camera or otherwise, or at right angles, by means of a Beale's camera lucida (see p. 112), to a piece of paper placed on the table, the microscope being raised, if necessary, to the requisite height so as to get the exact distance of 10 inches. Short-sighted observers may therefore need to use spectacles for this method. The magnification as seen by such observers when looking through the microscope will, of

course, be rather less than that calculated by the above methods, as explained on p. 131.

Magnification of Oculars.—The **Eyepiece Magnification** is readily calculated by dividing the combined magnification by the initial magnification of the objective independently determined. We may remind the reader, as explained earlier (p. 142), that the eyepiece magnification remains constant, but that if the tube be less than the normal 10 inches in length the initial magnification will be proportionately less, and the combined magnification will vary accordingly.

Magnification of Simple Hand-Lens.—The easiest way to measure this is to take a small object of known size, say $\frac{1}{2}$ inch or $\frac{1}{4}$ inch long, and to lay an ordinary rule on the table. The small object is then looked at through the lens with one eye, whilst the other eye looks beyond it at the rule, so that the two images are superimposed. As the normal visual distance is 10 inches the eye must be placed at this distance from the rule. Then if the object, say, of $\frac{1}{2}$ inch in length, when examined through the lens with one eye, covers 3 inches of the rule, looked at through the other eye, the magnification of the lens will be six times, or 6 diameters, and so on. It must be borne in mind, however, that if the observer be short or long sighted, he will require to put his eye nearer to or farther from the rule to see it distinctly, and therefore the lens *for him* will magnify proportionately less or more.

Numerical Aperture.—The **Numerical Aperture** is best measured by means of Abbe's apertometer. It is a plate of glass $\frac{1}{2}$ inch in thickness, and rather more than semicircular in shape. The straight edge is bevelled underneath to an angle of 45 degrees, so as to deflect the light through a small aperture in a silver disc placed

at the centre of the semicircle. The latter is graduated on the edge with the requisite numerical apertures and the equivalent angular apertures in air, and round the semicircle slide two metal discs to act as indicators. The points of these latter are easiest read when directed inwards for objectives below 0·7 N.A. and outwards for higher apertures. In use the apertometer is placed upon the stage, and the aperture in the silver disc focussed with the objective to be tested, the aperture being made as central as possible, and the tube-length of the microscope being that for which the objective is corrected by the makers. The flame of a lamp is placed opposite and level with the apertometer. Any eyepiece can be used. Without altering the adjustments, the draw-tube is then unscrewed, and a low-power auxiliary objective supplied with the apparatus is screwed into the universal screw generally found at the bottom of the draw-tube. The draw-tube and eyepiece are replaced, and this auxiliary objective is then focussed by means of the draw-tube upon the back of the objective to be tested, so that the images of the indices before mentioned are clear and distinct. The appearance, if the lamp is properly adjusted—for which purpose it may be necessary to move it first to one side and then the other —is that of a band of light across the field. As the indices are moved round the semicircular glass plate, and in close contact with it, their points will be seen on this band of light, and they are to be moved until these points just touch the edge of the luminous band, where it passes beyond the field of the objective. It is best to adjust the draw-tube so that the points of the indices and the back of the objective appear equally distinct. The readings are taken from the upper edges of the indices, corresponding to the points, and their mean gives the

requisite numerical or angular aperture. With microscopes of the Continental size of tube it is necessary to use a low-power eyepiece without any diaphragm between the eye and field lenses, otherwise the field of the latter may be too small to enable the whole of the back lens of a wide-angled objective to be taken in, and with very wide back lenses the auxiliary objective in the draw-tube may not be able to take in the whole back of the lens. Though the apertometer is not difficult to use, it requires some practice before its readings can be relied upon as a true test of the N.A. of an objective, owing

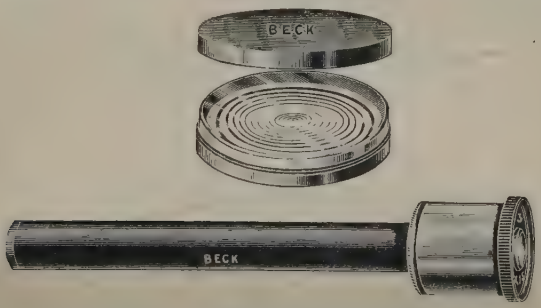


FIG. 104.—CHESHIRE'S APERTOMETER.

mainly to the difficulty of knowing when the points of the indices and the edge of the aperture of the objective are in actual alignment.

Mr. Cheshire has designed an inexpensive and easily worked apertometer, which gives very closely accurate results. It consists of a thick disc of optical glass, with a series of well-marked concentric rings on its lower surface (Fig. 104). It is only necessary to place this centrally on the stage, to focus a mark on the upper surface, to remove the ocular, and without disturbing the focussing adjustments, to look down the tube at the

back lens of the objective, and to count the number of rings seen there. Each ring denotes 0·1 N.A. A special ocular focussed by the draw-tube is provided for use with high powers to facilitate the counting of the rings. The apparatus is supplied by Beck.

Mr. Cheshire has also designed an even simpler arrangement, consisting of a diagram (Fig. 105), printed on card, together with a 25-millimetre cube of hard wood as a gauge for getting the correct distance of objective from

FIG. 105.—CHESHIRE'S CARD APERTOMETER.

the card, and a stop to drop into the eyepiece. It can, of course, only be used with dry lenses. A card apertometer on a slightly different principle is also made by Beck.

Penetration.—**Penetration** in an objective is the power of seeing deeper into an object than the exact plane nominally in focus. The accompanying diagram (Fig. 106) will show that the narrower cone of rays embraced by a narrow-angled objective gives a smaller angle in focus, and therefore permits of a greater depth of focus, if a less

accurate one, and one with less detail or resolution. It will be apparent that it is the converse of both aperture and power, being inversely proportional to both, and it is also dependent upon the size of the cone of illumination. The lower the power and the lower the N.A., the greater will be the penetration, but at the expense of the resolution. When dry lenses are used the penetration is also increased in direct proportion by using a medium of high refractive index in which to mount the object. By reference to Fig. 107 it will be seen that two planes at A and B are visible in the direction *a* and *b*, if mounted in air, but if mounted in a liquid of high refractive index

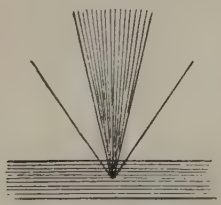


FIG. 106.—PENETRATION: EFFECT OF APERTURE.

two new planes deeper down come into focus at A′ and B′, and these are wider apart, and yet look as if they were situated at A B, higher up and closer together. They also form a narrower cone of rays, as in Fig. 107, and so give, in addition, a smaller point in focus with similar greater depth of focus.

The shutting down of the iris diaphragm beneath the stage will, of course, increase the penetration by decreasing the aperture of the objective, but the deterioration of the image is marked (see p. 173); and after all the proper way to get ' penetration ' visually is to use the fine adjustment, and to keep re-focussing in different planes.

A convenient way of increasing the penetration of an

objective for photography, where the penetration is least, owing to the absence of the accommodative powers of the eye, is by placing an iris diaphragm immediately behind it, and so reducing the aperture (see p. 121), but this tends to render the illumination unequal, unless the diaphragm be placed close to or in the objective.

Illuminating Power.—Illuminating Power, or brightness of image, other things being equal, increases as the square of the N.A., but decreases as the square of the magnification, owing to the smaller field. The increased amount of light due to a sub-stage condenser, or to

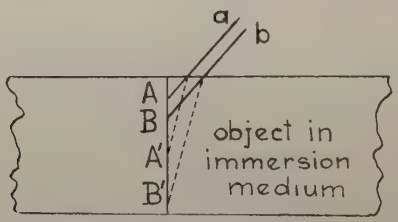


FIG. 107.—PENETRATION: EFFECT OF HIGHLY REFRACTIVE MOUNTING MEDIUM.

different sources of illumination, is, of course, another matter altogether.

Flatness of Field.—Flatness of Field varies much with different objectives, and perfect flatness of field is not obtainable with our present wide-angled objectives, owing to unavoidable spherical aberrations still outstanding. In practice its absence means that an object is not in focus as it approaches the margin of the field of view, but requires slight re-focussing to make it come sharply into focus. It is quite a different thing from a want of correction, which gives bad definition at the edge of the field of view, in spite of re-focussing. A spine of Echinus is often used as a test, but a micrometer slide is

preferable, and it will be seen that when the centre is in focus the lines become more or less hazy, and even curved outwardly, as they approach the periphery. The estimation of flatness of field is largely a manner of comparison. We may add that the eyepiece may be responsible to a certain degree, but this is easily determined by changing objectives and eyepiece.

Definition.—Defining Power is generally distinguished from resolving power, the latter being treated as a fixed quantity theoretically dependent on the N.A., as before explained, whilst the defining power is dependent upon the skill of the optician in calculating and making his lenses, upon the reduction of the spherical and chromatic aberrations, upon the exact centring of the lenses to their optic axis, and the accuracy with which they are placed at right angles with this same optic axis. One of the drawbacks incidental to the use of a correction collar is the difficulty in keeping in exact alignment the various lenses making up a combination. Defining power can only be decided by examining suitable test objects under critical conditions, and is a matter requiring much practice and experience. For suitable test objects, see p. 194.

Working Distance.—Working Distance is the free space, whether of air, water, or oil, between the front lens of the objective, or its surrounding brasswork, and the cover-glass of the object. It generally decreases, as might be expected, with increase of aperture, but varies in different objectives of equal aperture according to the skill of the computer and optician. It is always much less than the focal length of an objective. With high-power lenses it becomes an important consideration, and more so with dry objectives of $\frac{1}{8}$-inch and upwards than with immersion lenses. With a dry

$\frac{1}{6}$-inch objective of fairly high aperture (say N.A. 0·85) the working distance may be about 0·4 millimetre; with a dry $\frac{1}{8}$-inch of the same aperture it may be 0·25 millimetre or 0·15 millimetre; whilst a $\frac{1}{12}$-inch of N.A. 1·25 might have a working distance of 0·2 millimetre. It will be seen that cover-glasses of moderate thickness only should be used for objects that may be required at any time to be examined with high powers, but we have had objectives submitted to us for examination that would not focus through cover-glasses of even moderate thickness. If the milled head of the fine adjustment of the microscope be graduated, and the value of the screw known, the determination of the working distance of a high-power objective is an easy matter; it has only to be brought very carefully into contact with the cover-glass, and then raised until the object is in focus, the revolutions of the milled head being carefully noted, and the calculation made accordingly. Failing such knowledge of the pitch of the screw, a rough measurement can be gained by looking between the objective and cover-glass at a bright light, by using a carefully-made wedge, or even by measuring from temporary marks on the bearings in which the fine or coarse adjustment slides.

Diameter of Field—Size of Field.—This should be primarily dependent upon the power of the objective, irrespective of the aperture, but it is also qualified by the diameter and magnifying power of the eyepiece, and by the size of the diaphragm in the latter, this being smaller as the ocular increases in power. One advantage of the English size of tube is the increased field given with low-power oculars, and the firm of Zeiss endeavours to overcome this difficulty, not by increasing the diameter of the inner tube, but by making special

eyepieces to screw into the outer tube when the draw-tube is removed. Roughly speaking, an inch objective, with a moderate eyepiece, will take in a field 3 millimetres in diameter, a $\frac{1}{2}$-inch will give a field about 1·5 millimetres, a $\frac{1}{6}$-inch about 0·5 millimetre, and a $\frac{1}{12}$-inch about 0·25 millimetre in diameter.

Resolution and Aperture.—According to Professor Abbe, the 'resolution' of an objective—that is, its power of dividing a certain number of fine lines per inch—is dependent upon the obliquity of the rays of light to the axis of the microscope (and not upon the obliquity of the rays to the object, as was formerly supposed), which in turn depends upon the aperture of the objective.* It is therefore directly proportional to the N.A. The number of such lines to an inch resolvable by an objective with *axial illumination* and monochromatic blue light is obtained by multiplying its N.A. by 80,000. For daylight multiply by 70,000. If we multiply twice the N.A. by the wave-frequency of the light used, we arrive at the number of lines theoretically resolvable by *oblique* light. Add to this the fact that the normal eye can perceive about 200 or even 250 lines to the inch, and we get a ratio of aperture to power on which to base our calculations. The wave-length of ordinary white light varies from about 39,000 to 64,000 to the inch (0·75 μ to 0·35 μ). The average wave-length may be taken as 47,500 to the inch, and 1·4 N.A. the highest obtainable numerical aperture of a $\frac{1}{12}$-inch oil-immersion, therefore with oblique illumination $47{,}500 \times 2 \times 1{\cdot}4 = 133{,}000$ (say 0·2 μ), or with axial illumination, $70{,}000 \times 1{\cdot}4 = 98{,}000$ (say 0·25 μ), and these, therefore, may be taken as our present limits of *resolution* of any

* This remark does not profess to be an exact statement of Abbe's theory.

two lines or dots with ordinary light and oblique or axial illumination respectively. Moreover, they can only be increased by reducing the wave-length of the light used, either by monochromatic illumination in the green, blue, or even ultra-violet end of the spectrum; by shortening the wave-length by means of mounting media of high refractive index; or by increasing the aperture of our lenses. These statements must be carefully borne in mind in reading the next paragraph, on high magnifications, but it may be added here that, according to Lord Rayleigh, lines as narrow as $\frac{1}{16}$, or even under some conditions $\frac{1}{32}$ of a wave-length may be *visible* as such, and that there is no limit to the visibility of an object shown as a mere disc of light by a beam at right angles (see p. 161), other than want of sufficient light. But the mere making visible of an object as a vague disc, and the separating or ' resolving ' of lines close together are two different things (*cf.* p. 162). It follows that there is no advantage in mere magnification without increase of resolution, provided the magnification is sufficient to enable the observer to *see* what is required. This partly explains the disuse of the old high-power lenses, such as $\frac{1}{25}$- and $\frac{1}{50}$-inch, which gave mere empty magnification without increase of aperture. In fact, the small size of the front lens of such objectives, which the required magnification necessitates, carries with it an actual reduction in aperture, combined with most inconveniently short working distance, and the use of very thin cover-glasses. The author has personally never found any higher power than a $\frac{1}{12}$-inch—not even a $\frac{1}{16}$-inch—of any assistance in elucidating really difficult structures. The tendency of a beginner is to use high powers unnecessarily, but the practised worker invariably uses the lowest power

that will show him clearly what he wishes to see. The phrase 'a powerful microscope' is, of course, merely ridiculous.

High Magnification.—The fact that *resolution* in an objective depends entirely on aperture has already been explained, and as it has been mentioned that the eye can resolve lines from 200 to 250 to the inch, it follows that a comparatively moderate *total* magnification will make evident to the eye all the structure that the objective is capable of showing. For instance, a numerical aperture of N.A. 1·4 represents at the present time the highest aperture of the best oil-immersion apochromatic objective made, a lens costing nearly £20. This can be obtained as a $\frac{1}{8}$-inch or a $\frac{1}{12}$-inch lens. Now, an objective of N.A. 1·4 has been shown to be capable of resolving, say, 133,000 lines to the inch with oblique light, so that a total magnification of 665 ($\frac{133000}{200}$) is all that is actually necessary. A $\frac{1}{8}$-inch objective corrected for the 10-inch tube will have an initial magnification of 80, and such an objective used with a ×8 ocular will give a total magnification of 640, or with a ×9 ocular of 720. So that even with the high aperture of N.A. 1·4 a $\frac{1}{8}$-inch objective in a 10-inch tube and a ×8 or ×9 ocular will show all that our very best objectives *can* show, and therefore with our best objectives a total magnification of 720 is about as high as we need to go. This works out at about 0·2 of N.A. for each 100 of combined magnification for all objectives. The importance of realizing this is manifest.

At the same time it is a fact that many workers, we may even say the great majority, would not see such fine structure under such conditions without some training in looking for it. They require the image to be on a larger scale, they need a larger type, so to speak, or it

will not catch their attention, and so the use of a somewhat higher magnification is generally called for. This can be obtained by increasing the power of either objective or ocular, but it must be well understood that with the increase of power no more structure will be made evident; the image will be a little larger, but it will be less distinct, at any rate to the trained eye.

High-Power Oculars.—There has been much discussion on the question as to whether the additional magnification should be put into the objective or ocular. The practised worker will choose the highest aperture and the lowest powered objective and ocular that will show him what he wants to see, but the ordinary worker will often get more satisfaction out of a somewhat higher powered objective, than from a higher powered ocular. Of course, the penetration will be less, and so will be the field of view and the working distance, but the use of high oculars is attended by certain marked disadvantages. They, of course, magnify proportionately all the outstanding errors of the objective, which are practically no higher in a $\frac{1}{12}$-inch of N.A. 1·3 than in a $\frac{1}{8}$-inch of the same aperture, and incidentally the former is easier, and therefore cheaper, to make. Only the very finest lenses will stand eyepiece magnification even as high as ×10, and this disability increases with the highest powers. Even the $\frac{1}{8}$- and $\frac{1}{12}$-inch apochromats just referred to show a sensible falling off in the sharpness of their detail if used with a ×10 ocular on really difficult structure. Then the amount of light transmitted to the eye by an eyepiece depends upon the diameter of what is known as the ' Ramsden circle,' or ' eyepoint,' the disc of light above the eye-lens where the eye is placed, and which itself depends upon the N.A. of the objective and its magnification. With high-

powered oculars this disc becomes seriously reduced until it measures $\frac{1}{30}$ or $\frac{1}{50}$ of an inch, and as the pupil of the eye may be $\frac{1}{8}$- or $\frac{1}{10}$-inch in diameter it follows that the retina is insufficiently illuminated. This alone manifestly causes a disproportionate loss of light, and is one of the most evident drawbacks noticed on changing to a higher eyepiece. This loss of light takes place earlier with high-powered objectives than with low ones, owing to the narrow cones of light which they transmit. Incidentally, the narrow angle of the pencil of light falling on the retina reduces its power of separating fine structure, for which a physiological explanation is forthcoming. Moreover, the narrow pencil of light not only causes every speck of dust in the eyepiece to appear as a well-marked shadow, but also exaggerates the presence of physiological opacities in the vitreous humour of the eye known popularly as ' floating flies.'

It is, therefore, best to gain magnification by a proportionate increase in the power of the objective, until the highest apertures possible are reached, when we have no other resource than to use high oculars.

The Long and Short Tube Microscope.—In connection with this subject may be mentioned the question of the respective advantages of the long and short tube in getting a required magnification. It may be granted that the short tube is more convenient, especially when used vertically, and the fact that nearly all objectives are now turned out corrected for the short tube practically settles the matter beforehand, but it is often asked whether it is better to get a total magnification with a short tube and a higher eyepiece, or a long tube and a lower eyepiece. To give a concrete instance, does an apochromatic $\frac{1}{12}$-inch of N.A. 1·4 corrected for a 10-inch tube, and used with a $\times 6$ ocular, perform better than a

similar lens corrected for a 6-inch tube, and used with a ×10 ocular? Both give a combined magnification of 720, and the resulting image is in theory equally perfect, as the lesser magnification of the short tube gives a smaller and more perfect image to be subsequently magnified by the ocular, but Mr. E. M. Nelson, who has done so much for microscopy in this country, and whose skill as an observer and keenness of vision render him perhaps our foremost authority on the subject, avows his preference in practice for the results yielded by the long tube, going so far as to say that 'it must always be the ultimate court of appeal concerning any very minute structure,' and this coincides with the experience of the present writer. Incidentally it may be noted that the objective corrected for the short tube is more sensitive to errors or disturbance of correction, whether due to the perfection of adjustment of the objective itself as sent out by the optician, a matter in which there is sometimes more variation than might be imagined, or to disturbances set up by varying thickness of cover-glass, variations in tube-length, etc. Mr. Conrady, whose opinion carries great weight, considers, however, that there is really no observable difference in the two images, and once observed to the writer that an incidental disadvantage in getting objectives specially corrected for the long tube lay in the fact that in the final corrections and touches given to an objective the personal element entered not a little, and that a man who adjusted many batches of objectives for the short tube might, without reflection on his skill, succeed less perfectly with the comparatively unaccustomed correction for the long tube.

Optical Index.—Mr. E. M. Nelson has suggested that a new standard should be adopted which would show

the value of an objective both with regard to power and N.A., and that accordingly the N.A. should be multiplied by 1,000, and divided by the magnifying power of the objective, the result being entitled the ' optical index.' It would be well if the term could come into general use.

Theoretically, objectives should have 0·26 N.A. for each 100 diameters of combined magnification (that is including the eyepiece), if they are to be capable of showing all that is theoretically possible, but in practice very much less than this suffices. Some makers, for instance, make most of their objectives of moderate aperture, not only because they are less expensive, but because they give more of what is known as ' penetration,' or the power of seeing more than lies in one actual plane. Moreover, a high aperture reduces the working distance. The tendency of the day is, however, as has already been said, to insist on high apertures, and each maker tries to outdo his competitors in this respect. Our advice to beginners would be to content themselves with moderate apertures—at any rate at first—but, above all, to get objectives made by first-rate houses only. If of other than English make, see that the screw is of the Society gauge (see Appendix), though this is now well-nigh universally adopted.

Under- and Over-Correction.—This can be estimated by examining a coarse, unstained object by either axial or oblique illumination. All achromatic objectives will give a certain fringe of colour, which is increased if the condenser is not properly centred. With axial illumination if the objective is under-corrected, there will be a bluish fringe, which will be increased when it is slightly racked up out of focus, whilst there will be an orange fringe when it is racked within the focus. When the mirror is swung to one side there will be a blue

fringe on the side to which the mirror is swung, and an orange fringe on the other side. Over-correction shows exactly opposite results with both methods.

Aplanatic Aperture of Condenser.—It has already been stated (p. 90) that the true value of a condenser lies in its **aplanatic aperture,** or in the proportion of the condenser's total aperture which is free from spherical aberration, and this may be estimated approximately as follows: An object mounted in Canada balsam is placed upon the stage of the microscope, and the objective being first focussed upon it, the condenser is then centred and focussed as described on pp. 174 and 176, using the edge of the lamp flame, and focussing it in the centre of the field. Without altering the adjustments the eyepiece is then removed, and on looking down the tube, if the whole of the back lens of the objective appears filled with unbroken light, the aplanatic cone of light transmitted by the condenser exceeds, or at least equals, the numerical aperture of the objective used. Within narrow limits a slight racking up of the condenser not infrequently assists the determination, but the appearance of two dark spots shows either that the condenser has been racked too high or that its aplanatic aperture is lower than that of the objective. In this case an objective of less aperture must be tried, or, in the converse, one of higher aperture. The result may be invalidated if a slide of unsuitable thickness be used, or the lamp be placed too far away.

Definition of Condenser.—The definition of a condenser can be roughly tested by using it as objective, and screwing it into the nosepiece of the microscope, but, of course, this only applies to small-sized condensers fitted with the objective standard screw. As the condenser is corrected for use through a slide, the latter

must be placed on the stage upside down. Definition comparable to an objective must not be expected.

The Limits of Vision and of Resolution.—The present is a convenient place in which to deal with two matters which were brought forward by the firm of Carl Zeiss (and followed by Leitz and Reichert) since the first edition of this book was published, and which raised extravagant expectations amongst workers with the microscope. These relate to apparatus for demonstrating 'ultra-microscopic particles,' and to photomicrography with ultra-violet light.

Ultra-Microscopic Particles.—Though Abbe made clear the dependence of 'resolution' upon 'aperture,' and the practical limitation of such *resolution* to our present limitations of aperture, he agreed with Helmholtz as to there being no limit of *mere visibility*. This has been well expressed by Lord Rayleigh as follows: 'In the microscope there is nothing except lack of light to hinder the visibility of an object, however small. But if its dimensions be much less than half a wave-length, it cannot be seen as a whole, and its parts cannot be distinctly separated, although in cases near the borderline some inference may possibly be founded upon experience of what appearances are presented in various cases. What has been said about a luminous point applies equally to a luminous line. If bright enough, it will be visible, however narrow; but if the real length be much less than the half wave-length, the apparent width will be illusory.' It is, perhaps, advisable to emphasize the distinction between the mere making visible of an object and the seeing of it as it really is, so that it can be seen and studied.

The theoretical limit of resolution, according to the Helmholtz and Abbe theories, is about 0·25 μ, and

therefore, if a particle has a dimension of less than half a wave-length of the light used, we must expect a microscopical image of such particle to be merely a diffraction disc, which, whilst certainly demonstrating the existence of such a particle, gives no intimation of its actual structure. Such necessarily imperfect images are spoken of as 'ultra-microscopic' particles, and there is no limit to the size of such particles which can be demonstrated other than the impossibility of obtaining a sufficiently powerful illuminant, not excepting direct sunlight. Very minute particles are found, for instance, in gold ruby-glass, and Dr. H. Siedentopf, working in conjunction with Dr. R. Zsigmondy, designed a new form of dark-ground illumination which, whilst illuminating the particle so strongly as to cause it to diffract a visible cone of light rays, at the same time excludes from the eye all other light, which would otherwise overpower such a diffraction image, and, further, obviates the innumerable reflections that occur at the surfaces of the several lenses of the condensers, or in the object itself, when the usual dark-ground illumination is made use of. For instance, as Dr. Siedentopf puts it, small particles of dust become visible in the air when a beam of sunlight enters a dark room through a hole, provided the observer's eye be approximately at right angles to the beam. Briefly, therefore, in the apparatus designed by Dr. Siedentopf, light from an arc-lamp is focussed upon a small spectroscopic slit which can be adjusted so as to pass a narrow beam of light through another condenser upon the particles to be made visible. This beam of light must be at right angles to the cone directed upwards into the microscope, and the thickness of the illuminated layer must correspond to the depth of focus of the objective—

say 1 to 3 *u*. By this means diffusion of light from planes, other than those in focus, which would otherwise form a disturbing veil of light, is eliminated by illuminating only those particles which are to be made visible. By this method, allowing for the limit of sensitiveness of the human eye, Dr. Siedentopf works out a visibility, apart from any question of shape or size, of a circle with a radius of about 0·000004 millimetre. By grinding flat and blackening a small central portion of the curved surface of the front lens of an objective, to suit its aperture, Dr. Siedentopf has obtained further results on bacteria, which made him believe in the feasibility of viewing 'ultra-microscopic' bacteria, 'so that bacteriologists may actually discover germs which have been suspected to exist.' It may be added that he recognizes that, accepting the size of a medium-size molecule as 6 $\mu\mu$, a specific intensity of luminosity considerably exceeding the power of the sun's rays would be necessary to discern such molecules.

The apparatus has been arranged also for the investigation of colloidal solutions. It is made by Carl Zeiss, who first introduced it, and in a modified form by Leitz and Reichert. The ingenuity of the apparatus is undeniable, but its limitations are evident, and so far its use has not added much to our knowledge of structures which are beyond microscopic vision as ordinarily understood.

Photo-Micrography with Ultra-Violet Light. — The resolving power of a lens depends upon its aperture, and with cedar oil as an immersion medium we are limited to a N.A. of about 1·4. An immersion lens has been constructed for use with monobromide of naphthalene which has a N.A. of about 1·6, and proportionately greater resolving power, but this medium

is, unfortunately, unsuitable for mounting most objects, so that this lens has not come into general use. But we can increase the resolution in two other ways, as already mentioned (p. 154)—either by increasing the refractive index of the mounting medium, and so shortening the wave-length of light, or by using light of shorter wave-length. White light is known to be made up of rays of different wave-length and refrangibility, those at the violet end of the spectrum being much shorter than those at the red end. Monochromatic light, selected by means of a prism or screen, and taken from the blue, or, still better, the violet end of the spectrum, will consequently give us greater resolution than ordinary white light, which contains so many rays of longer wave-length. This is taken advantage of in photo-micrography, and the result is a very definite increase in resolution—say, of the markings of a difficult diatom. Unfortunately, the eye itself is but little sensitive to such rays, so that it is not easy to see the object directly, or to focus it, though such light has high actinic value. Dr. Köhler, of Jena, has, however, experimented with ultra-violet rays of a still shorter wave-length—275 $\mu\mu$—which have even greater power of resolution, but are, of course, invisible, and has had lenses made of crystal and fused quartz corrected for this wave-length, which, as monochromatic light is used, have the incidental advantage of needing correction only for spherical, and not for chromatic, aberration. The N.A. of the strongest system is 1·25, which gives a resolving power, as compared with an imaginary ordinary lens used in daylight, equivalent to N.A. 2·5, on account of the small wave-length of the light used. The light itself is obtained from a 2-millimetre spark between cadmium electrodes of a Leyden jar, worked with an induction

machine, and light of the required wave-length is separated by an iris diaphragm and passed through a condenser made of quartz.

But the human eye, as has just been stated, cannot see these rays, and so cannot focus and adjust them. Therefore Dr. Köhler devised what may be called an artificial eye; in other words, he constructs what corresponds to the lens of the eye—*i.e.*, an eyepiece, made also of quartz, and a retina or screen made of fluorescent glass, which responds to the ultra-violet rays. The image on this screen is examined visually by means of an ordinary magnifying pocket 'lupe,' in which case Dr. Köhler found magnesium light, having a wave-length of 280 $\mu\mu$, more suitable than cadmium light. This lens, impervious to the injurious ultra-violet rays, thus renders them visible for rough examination and focussing. A photograph is then taken, showing the finer detail. It is to be noted that these quartz lenses can only be used with light of the wave-length for which they are constructed, that an immersion medium of suitable refractive index (made of glycerine and water in proportions calculated to give such a definite refractive index) must be used, and that the cover-glasses and slides must also be made of fused quartz, or the latter can be made of ultra-violet permeable glass. An ordinary vertical camera is used, but the distance between plate and ocular must be approximately 30 centimetres, varying slightly according to objective and eyepiece used. Three objectives are made—6 millimetres of N.A. 0·35, 2·5 millimetres of N.A. 0·85, and 1·7 millimetres of N.A. 1·25—giving relative apertures, as compared with ordinary objectives, of 0·7, 1·7, and 2·5 respectively. Five eyepieces of magnification varying from 5 to 20

are also made. The price of the objectives is, unfortunately, high, varying from £10 to £30, and the total outfit, not including the microscope and induction coil, would cost from £80 to £110.* Its cost, therefore, its limitation to photography only, and its special requirements in mounting, render its uses limited, but we mention it here at so much length because it represents an attempt on thoroughly scientific lines to increase our means of investigating minute structure, and in order that its possibilities, as well as its limitations, for the ordinary worker may be thoroughly grasped. The apparatus is made by Carl Zeiss.†

Mr. J. E. Barnard, in the recent work on cancer done by Dr. Gye and himself in collaboration, introduces an ingenious arrangement by which a special dark-ground reflecting condenser (see p. 95) is used to form a dark ground and visible image with monochromatic green light from a mercury vapour lamp. But the centre of this condenser, instead of being blocked out, contains a quartz lens condenser with which to focus direct rays of ultra-violet light from a cadmium spark-gap, the two types of condenser being thus combined in one mount with the necessary change-over diaphragms included. The visible indirect refracted light and invisible direct ultra-violet light, by reflection from a prism, are used alternatively as required. Thus a diffuse refraction image is observable, but the actual photograph is taken with ultra-violet light of 275 $\mu\mu$,

* These are pre-war prices.

† A full description of the apparatus appeared in the *Journal of the Royal Microscopical Society* in 1905, pp. 513-518, and of the principles (on which the preceding paragraphs are based) on pp. 103-104. The author has left the above paragraphs practically unaltered, as they were written in 1909, in justice to Dr. Köhler as the pioneer in photo-micrography by ultra-violet light.

or even 257 $\mu\mu$, with consequent gain in resolution (see p. 164). Quartz lenses and oculars corrected for this wave-length, as used by Dr. Köhler, are, of course, necessary. It has certain advantages over Dr. Köhler's method by eliminating the use of a fluorescent screen for the preliminary observations and rough focussing. Mr. Barnard's apparatus is made by Beck.

It will be noted, however, that the use of such an apparatus is strictly limited, requires skilled adjustments, and in no way replaces ordinary microscopical work. Visual work on the observation of particles too small for resolving, but not too small to see as diffraction discs, requires quite simple apparatus, which can be used in an ordinary microscope, and has been already dealt with on p. 94.

Gordon's Oscillating Screens.—There remains yet another attempt at increasing our powers of microscopic vision to which reference must be made. The use of high-power eyepieces beyond a certain point in order to gain magnification is attended with several disadvantages, already mentioned on p. 156, especially serious loss of light owing to the smaller angle of light-rays which they can collect, and the very narrow pencil of light which they can transmit through the pupil of the eye, with accentuation of shadows thrown by dust on the eyepiece, or imperfections in the vitreous humour of the posterior chamber of the eye. Mr. J. W. Gordon has dealt with this by focussing the image on a finely-ground glass screen, and then magnifying this image by a second microscope, magnifying as much as 100 diameters. To obviate the magnification of the grain of the ground glass, the latter oscillates in an excentric movement. This ingenious arrangement gives a much improved image, but it is necessary carefully to bear

in mind that the fundamental principles of microscopic vision remain true, and that as resolution is dependent upon aperture, so quite moderate eyepiece magnification will show all that an objective can make evident (see p. 153), and therefore further magnification, however well carried out, and however clear the resulting image, is but mere magnification of an existing image, and shows no further structure, just as a negative will bear only a limited enlargement in an enlarging camera. Mr. Gordon's method, accordingly, though ingenious, is not likely to lead to further discovery in structure. It is necessary to utter this warning, as so much ill-informed speculation appeared in print as to what we shall be able to discover with the new magnifications of 10,000 and more !

CHAPTER VII

MANIPULATION OF THE MICROSCOPE AND ITS ACCESSORIES

It is necessary now to deal with the management of the microscope and its accessories. The modern microscope has become an instrument of such exquisite precision that the beginner generally finds himself at the very outset in need of explanation and assistance in this respect. A clear understanding of what he is doing is necessary if he is to obtain the best results. In our medical and other laboratories too little attention is often given to these matters, and it follows, therefore, that the student is not really familiar with the use of his instrument, and obtains more or less imperfect results. The general use of the sub-stage condenser has revolutionized microscopy, and attention must again be called most strongly to the fact that the proper management of the illumination of the object is a matter requiring as much or even greater care and attention than the actual focussing of the objective itself. This last is comparatively a simple matter, save where corrections have to be made for different thicknesses of cover-glasses.

Setting up Microscope.—Let us assume that the reader has provided himself with a microscope fitted up in accordance with the rules and suggestions made in Chapter III. He will place his microscope on the table before him, and incline it, if possible, at a comfortable angle for looking down the tube. Of course, there is

a certain amount of work that can only be done with the microscope in a vertical position; but when the object will admit of it, it is a great convenience and rest to the muscles of the neck if the microscope be slightly inclined. This is a point that makers of even the less expensive foreign microscopes are beginning to realize, as one by one English improvements are adopted on the Continent. It will be found that ordinary daylight, though perfectly suitable for low-power work, is totally unsuitable for high-power and *critical* work, and as we are setting up our microscope for critical work, we will deal with lamp-light illumination accordingly. The beginner need scarcely be warned that the use of direct sunlight is absolutely out of the question for visual purposes. The lamp will be probably an ordinary ½-inch paraffin lamp, with a shade, as already described, but without a reflector, as this last only succeeds in confusing the rays of light. Some workers place the lamp on the immediate left of the instrument, in order to obviate any glare into the eyes, but the position immediately opposite and in front has so many advantages in ease of manipulation that this position is generally preferred, with a cardboard shade interposed if required. The lamp will therefore be placed in the latter position, with its wick turned *edge-ways* forward, the light being about 6 to 10 inches or thereabouts from the mirror, as most achromatic condensers are corrected for light at about this distance. It is well to accustom oneself to these approximate distances, as high-power immersion condensers are adjusted, amongst other things, for light at a definite distance from their back lenses. It will be necessary to observe carefully at the outset that the flame is immediately opposite the centre of the stage, and that

the tail-rod and the mirror are approximately truly in line with the optic axis of the microscope. This is important. A slight tilting of the mirror will then be sufficient to fill the tube with light. The beginner can with advantage make his first essays with both objective and eyepiece removed. For an objective lower than $\frac{1}{2}$ inch or $\frac{2}{3}$ inch the condenser will generally not be required, and in this case the concave mirror and the flat side of the flame can be used. The concave mirror should be adjusted at such a distance from the object on the stage that the rays of light are approximately focussed on the latter. A little reflection will show that when parallel or slightly divergent light falls on a concave mirror the light becomes *convergent*, and the focus depends mainly upon the curve of the mirror itself. If the condenser be retained for these low-power objectives, the upper lens should be removed, or the field of illumination becomes inconveniently circumscribed; but for the reasons just given *the plane mirror must always be used with the condenser*. We have not infrequently seen workers, otherwise experienced, using the concave mirror with the condenser because of the increase of light obtained by the use of the concave mirror *without* the condenser, but forgetting that by so doing they are throwing upon the condenser convergent instead of nearly parallel light, and making it impossible to focus satisfactorily the condenser itself.

Focussing Objective.—The beginner will be wise to bring his objective close to the slide, by examining it from the side, and then to focus *up* so as to be out of danger, but it may again be advisable to point out (see p. 140) that the so-called focal length of the objective does not in any way represent its distance from the

cover-glass of the object. In fact, with increase of aperture the objectives have got closer and closer to the object. A safe way of using immersion lenses is to focus some dust on the cover-glass, or the edge of the cover-glass, first. An excellent way, when the size of the stage opening permits, is to slip the tip of the finger under the slide so as to lift it slightly, when any pressure from the objective can be at once felt, and the slide is then lowered into its proper position, and re-focussed with safety. The habit should be formed of always slightly racking objectives up after use, so as to be out of danger from the edge of the mount when rotating them on a rotating nosepiece.

The beginner is recommended to find out at the time of purchasing whether his objectives are corrected for the 6-inch or 10-inch tube, and to remember that they perform properly only on the tube for which they are designed. Most students' objectives, as already stated, are corrected for the short tube.

Use of Condenser.—For objectives above $\frac{2}{3}$ inch the use of the condenser is advisable, and for higher powers imperative, if the full value of the objective is to be realized. In Continental microscopes, until comparatively recently, a sub-stage condenser was the exception rather than the rule. In many of our English laboratories its use, therefore, is even yet scarcely understood, because of the curious prejudice in favour of the Continental stand; but though the Continental makers turn out excellent objectives, they have apparently only lately begun to realize that an objective of high aperture has really little advantage over one of low aperture unless used with a suitable condenser. The condenser sold with Continental stands is even now generally the Abbe illuminator, with its large spherical and chromatic

aberrations, and small aplanatic cone, and without centring adjustments.

The cone of light transmitted by the condenser should approximate to that of the objective if the latter is to perform at its best, but few objectives will stand solid cones of light equal to their own apertures. Even the apochromats, on difficult objects such as diatoms, will not stand a full cone, but become flooded with light, which causes a hazy image. If the worker is using the Abbe illuminator of 1·2 N.A., he will, of course, only obtain a total aperture of less than 1·0 N.A., unless the condenser is in immersion contact with the under side of the slide. Theoretically, therefore, this condenser is suitable for lenses of 1·0 N.A. and even more, and suitable also for lenses of lower apertures by the simple process of stopping the condenser aperture down by means of the iris or other diaphragm. The worker soon becomes expert in the use of the iris diaphragm, but beginners must be cautioned against the too prevalent tendency to cut off an unnecessary portion of the marginal rays in order to secure contrast in the image. By so doing the latter suffers rather than gains, diffraction images being set up. As a matter of fact, the diaphragm should be closed gently and cautiously, just enough to accentuate slightly the image and no more, and it will generally be found that, under these circumstances, if the eyepiece be removed, the back of the objective, examined down the empty tube, is found to be from two-thirds to three-quarters filled with light. A good objective should stand satisfactorily what is accordingly often spoken of as a three-quarter cone of illumination. For really critical work with high powers a fine adjustment to the condenser is of much service, as the slightest adjustment will often make all the difference in the image.

Focussing Condenser.—Having so arranged lamp and mirror that the object is properly illuminated, it will be necessary then to focus the objective upon the slide, and, having done that, to adjust the condenser up or down, until the image of the edge of the lamp flame appears distinctly in the centre of the field. The mirror may require adjustment for this. The flame should appear sharply contrasted with the rest of the darker field if the condenser is well corrected. The light is thus correctly adjusted, its rays being brought to a focus upon the object, and so entering the objective undisturbed. To a beginner the partially illuminated field seems strange, but he must remember that in a critical image it is necessary to get the maximum perfection of definition in that portion of the object immediately under examination, and the rest may be ignored. If, however, a larger field be required, the flame, having been focussed, can then be turned broadside on, without altering its other positions. For ordinary work this is quite usual, but less suitable for the highest class of critical work. With the ill-corrected Abbe illuminator the image thus obtained with the flat of the flame can scarcely be considered to be a 'critical' one.

In earlier days it was customary, after focussing the light, to rack the condenser either up or down until the field was fully illuminated, and it is said that the Quekett Club, which has done so much for amateur microscopists, held two vehement rival schools, one of which maintained that the condenser should be racked up, and the other that it should be racked down. All these matters have now been settled by the realization of the real principle, and even more, perhaps, by the improvement in objectives, by which, under such

illumination as now obtains, hidden structure has been brought to light that before was not even suspected. It may be well to warn beginners against racking the condenser so high as to drive the slide up against a high-power objective. The focus is very short—sometimes, indeed, almost touching the slide, and some condensers will not focus at all through a thick slide. Slightly greater working distance may be gained by bringing the lamp closer to the mirror. The high-powered condenser of, say, $\frac{1}{4}$-inch focus necessarily gives a small but very bright image of the illuminant, and is, therefore, suitable for high powers only. The brightness of the image is due to the small area into which the image of the illuminant is condensed, and partially counteracts the loss of light due to the high magnification of the objective. Well-corrected immersion condensers are necessary if critical work is being done with high-apertured immersion lenses. A drop of cedar oil makes the contact between condenser and slide, and care must be taken that immersion contact is maintained, and that the oil is free from air-bubbles.

The principle which underlies the use of the condenser amounts really to this—that the light and image rays must coincide. If the condenser be adjusted so that its focus be *beyond* or *below* the object on the stage, the rays of light will not coincide with the focus of the objective, but will cross, and the result will be an imperfect image.

There is one other point that may be mentioned in connection with the focussing of the condenser. So far it has been purposely assumed that the source of light has been that of a lamp, which gives slightly divergent light. In Dr. Carpenter's book on the microscope it is rightly pointed out that ordinary daylight,

owing to its diffusion, does not give parallel light, though a window acts to some extent as a limiting diaphragm. The focus of the condenser differs somewhat, therefore, for lamplight and for daylight. The bars of a window frame or a cloud may be used for focussing daylight, after which a slight movement of the mirror throws them aside, but, as previously stated, daylight is useless for critical illumination.

Centring Condenser.—The necessity of making sure beforehand that the mirror and tail-rod are truly in line with the optic axis of the microscope has been already alluded to (p. 171). If this be not the case, the image of the lamp flame will appear to shift in position as the tube of the microscope is racked up and down. It is necessary, in addition, that the condenser itself shall be truly centred with the objective. Microscopes fitted with a simple sub-stage ring are only approximately centred, but the mounting of the condenser is generally slightly elliptical, and by rotating the condenser an approximately central position can generally be obtained. The value of a proper centring sub-stage is now evident, and the simplest way of centring the condenser is to make a minute ink-spot in the centre of the top lens and centre accordingly, afterwards wiping the ink-spot off. Some condensers were formerly fitted with a small brass perforated cap for this purpose. The more usual method is to focus the image of the iris diaphragm when practically closed, and to centre accordingly.

The image of the iris diaphragm will only be seen when the objective is racked some distance upwards, and this is preferable to racking the condenser down, as the latter seldom remains in the same alignment with the objective under these conditions. With high powers it is often not possible to get an image of the

diaphragm small enough to centre by, and therefore the initial step is taken with a $\frac{3}{4}$-inch or less. The condenser being centred, the objective is focussed on the object on the stage, the condenser then focussed by bringing the image of the flame sharply into focus, and the latter again brought to the centre of the field either by adjusting the mirror slightly or by a small movement of the lamp. With a change of objective and consequent alteration of centring it is only necessary to bring the lamp flame back into the centre of the field *by adjusting the centring screws of the condenser, the mirror and lamp remaining untouched throughout,* so as to serve as a guide for the requisite new adjustment of the optic axis.

It may be advisable here to repeat that almost all objectives differ in the centring of their mounts, and therefore with high-power work the centring of the condenser will vary for each objective. A rotating nosepiece, as stated on p. 107, also disturbs the centring of the objectives, and consequently of the condenser, as no rotating nosepiece is constructed with sufficient accuracy to insure absolute truth in this respect.

Oblique Illumination.—In most cases strictly axial, *i.e.*, central, illumination is used, but there are certain cases—such, for instance, as the structure of diatoms—for which oblique illumination is necessary. Formerly this was obtained by swinging the mirror, or mirror and condenser combined, slightly to one side, but more generally now by the insertion of a stop in the carrier beneath the condenser. In this stop a narrow slot has been cut, or two slots at right angles, or a segment cut out. The effect of this is to throw an oblique beam of light in one or more directions across or down the diatom, in addition to the axial or dioptric beam, accord-

ing to arrangement, and thus to bring into greater prominence shadows of the fine markings, otherwise almost invisible owing to their exceeding minuteness. To do this properly requires considerable practice and experience, as may be readily understood when we remember that the ' markings ' in *Amphipleura pellucida* exceed 90,000 to the inch. The Continental form of rackwork lateral adjustment to the iris diaphragm is very convenient for this purpose.

Dark-Ground Illumination.—The use of oblique illumination by means of suitable discs placed beneath the condenser in a carrier adapted for that purpose has been just alluded to. There are also other forms of illumination generally spoken of as ' dark-ground illumination.' In the simplest case a small central spot, supported on arms, occupies a similar position (see p. 93), and by cutting off those central rays of light which would otherwise enter the objective gives an absolutely dark field in the microscope. When a suitable object is interposed on the stage, however, the annular rays of light that would otherwise escape the objective are intercepted by the object, and thus diffracted into view. The result is often singularly beautiful—diatoms, foraminifera, and transparent zoophytes being exhibited shining upon an otherwise dark and contrasting background. To obtain the best results it is necessary to bear in mind that the diameter of the spot must be proportioned to the aperture of the objective. Thus, a low-powered, and presumably low-apertured, lens will require a *small* spot, whilst a high-powered and high-apertured lens will require a much larger spot. It would not be of much advantage if we were to give methods for ascertaining the size of spot required; if necessary, an experimental spot can readily be constructed out of blackened card-

board. The Traviss expanding stop (p. 93) gives excellent results, especially if combined with a Davis shutter. The condenser will also require a certain amount of adjustment, and the Davis shutter, which is a small iris diaphragm fitted *above* the objective, by its facility in reducing the aperture of the objective, enables one to obtain perfect background illumination. With ordinary condensers dark-ground illumination is, however, only possible with objectives of moderate power and aperture, say, not exceeding 0·6 N.A.

Before leaving the subject of oblique illumination, it may be mentioned that mere slight tilting of the mirror will often greatly increase the resolution of difficult objects.

Dark-Ground Condenser.—The paraboloid and dark-ground condenser have already been discussed (see p. 94), and it has been explained that their general principle is that the object refracts light of a higher N.A. than 1·0, whilst the objective must therefore not take in light beyond this aperture, or the dark background becomes impracticable. For a large amount of work dry lenses are suitable, but immersion lenses give a much improved image, by obviating undesirable reflections from cover-glass, etc., and as being less sensitive to variations in thickness of the latter. But (except with the Cassegrain illuminator) the immersion objective must be provided with a removable stop to cut its aperture down as required. Moreover, centring screws are essential either to the sub-stage or to the condenser, or (less satisfactorily) to the objective (see p. 12).

The usual method of using the dark-ground condenser is as follows: The source of light may be a small arc lamp, a 'Pointolite' lamp, or a mercury vapour

lamp (see p. 118), and a bull's-eye condenser which focusses the image about 6 inches away from the back of the dark-ground condenser. The best way to obtain this is to focus on a piece of paper as described on p. 184, but as the microscope is generally used upright, the light will be adjusted in line with the mirror. These adjustments, especially that the mirror lies truly in the right axis, should be made carefully. Both condenser and objective are then removed from the microscope and a low-power ocular alone inserted, and the light coming through this examined about 10 inches away, the mirror being tilted until the bright ring of light, formed by the eyepiece diaphragm, is seen centrally. In all subsequent adjustments or changes of objective the mirror must now remain untouched.

The dark-ground condenser is now inserted, the slide put on the stage, immersion contact made between condenser and under side of slide, and the image examined with a low-power ($\frac{2}{3}$ inch) objective. The disc or ring of light now seen should be centred with the centring screws, *not* with the mirror. For this centring some makers engrave a tiny cross on the top lens.

The condenser should now be *focussed* by altering the focus until the small ring of light generally seen becomes the smallest possible disc of light. The crossing oblique rays of light from the condenser are thus focussed on the object.

The low-power lens is now exchanged for the immersion lens (with funnel stop to reduce the aperture), and if, as generally happens, there is a change in centring, the condenser is again centred by means of the centring screws.

The objects should now stand out brightly on a black background. In some cases a piece of oiled ground-

glass put between the mirror and the light will improve the image, but it must be remembered that the adjustments are critical. It must also be noted that the slide must be of the required thickness (see p. 95), that it, the liquid medium, and the cover-glass must all be parallel or diffuse images result, that the latter must be clean and free from scratches, and that both condenser and lens must have good immersion contact, *free from bubbles*. For immersion contact of the condenser Mr. Nelson recommends golden syrup as being more viscous and maintaining contact better, whilst to obviate want of parallelism in cover-glass and slide Leitz makes a special chamber with springs, and a hollow cell like that in the usual type of hæmocytometer.

It will be found that too much light tends to increased glare, and with dry lenses that any variation in thickness of cover-glass from that for which the objective is corrected tends also to diffuseness of image, and though this can be corrected to some extent by varying the tube-length, a correction-collar to the objective is a great help (see p. 136). High eyepiecing, often required, accentuates all these difficulties.

Reflected Light.—Opaque objects are illuminated by several methods. (1) The most frequent way is to focus the light directly upon the object by means of a **bull's-eye** stand condenser, remembering that the flat of the condenser must be nearest to the object and quite close to it, the focus being short. If a lamp be used, it will be necessary to raise it well above the stage. This is useful for low powers only, as the objective gets in the way with high powers. The illumination is distinctly one-sided, and there are marked shadows. (2) A better way is by the use of a **side silver reflector,** which is a small silver parabolic mirror placed close to the object (Fig. 104), and

reflecting the rays of light thereon from a lamp placed quite near and about level with the stage. Its management is soon learnt. The lamp rays may be reinforced by means of *parallel* light from a bull's-eye condenser, as explained below. The illumination is better and stronger than by the previous method, but still somewhat one-sided. The bull's-eye must be carefully centred. (3) Perhaps the most perfect means of illuminating opaque objects is by the now but little used **Lieberkühn.** This is really a speculum fitted above and round the objective, the light being thrown from *beneath* the object, and reflected down again upon it. Its disadvantages, and those which have caused it to be largely disused, are that each objective must be fitted with its own Lieberkühn, and that the object must be mounted, not upon a black background, but in such a way as to allow the light to pass up and around it. When dealing with mounting it will be pointed out that the generally recommended method of mounting opaque objects upon a black background is not only unnecessary, but inconvenient. The writer invariably mounts such objects in an ordinary cell, and puts under them a plain slide upon which a disc of black paper has been fastened. What more is needed? (4) As already stated (p. 105), the **vertical illuminator** (Figs. 65, 66) can only be used with *uncovered* objects, or those in which the object is in optical contact with the cover-glass and mounted dry. Immersion lenses may be used, and, indeed, it is the only means of illuminating opaque objects with such objectives. Some little attention to adjustment is necessary if the best results are to be obtained. The lamp must be placed on a level with, and exactly opposite, the hole in the vertical illuminator, and at a distance approximately equal to the distance from the reflecting

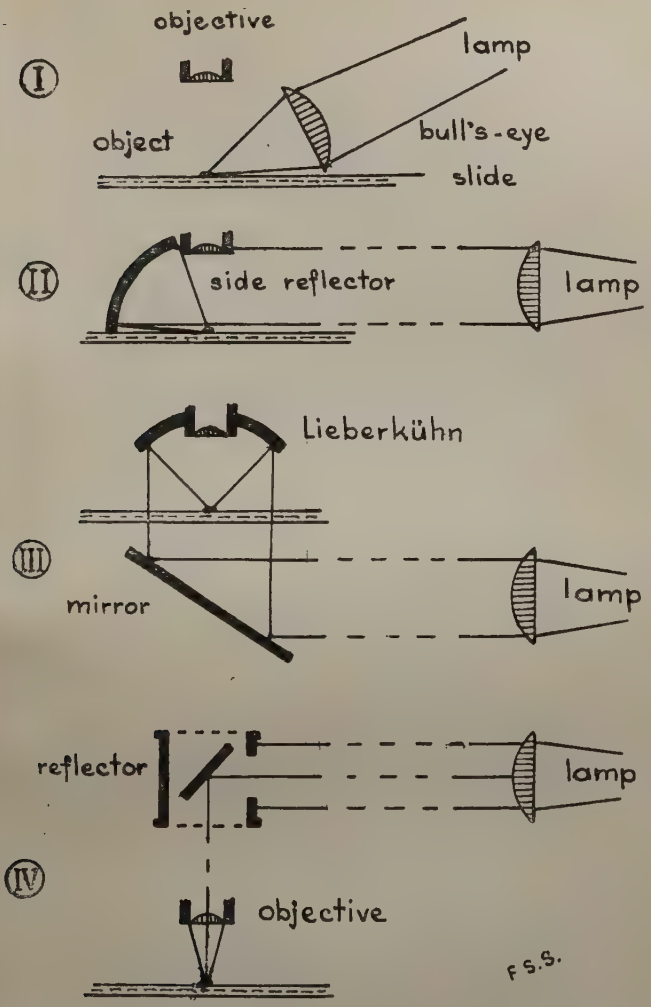


FIG. 108.—METHODS OF ILLUMINATING OPAQUE OBJECTS.

cover-glass or prism in the reflector to the diaphragm of the ocular—generally about 6 inches. The light must, of course, be carefully centred. If the cover-glass form of illuminator is used, the cover-glass must be set at an angle of exactly 45 degrees. The prism form passes less light to the eye owing to its cutting off half the aperture of the objective. There is generally a good deal of glare due to reflections from the reflector, and from the lenses of the objective, and these are most marked with dry lenses. The accompanying diagrams (Fig. 108) show roughly the position of the bull's-eye and lamp with each form of illumination.

Use of Bull's-Eye Condenser.—Before leaving the subject of illumination, the bull's-eye condenser must be considered. The great spherical and chromatic aberrations of this lens were alluded to in Chapter V., p. 102, and these render it unsuitable for really critical work on account of its bad definition. For this reason its general use cannot be advocated, save for opaque illumination. Should it be desirable, however, to fill the field with light by its means, without attempting to attain a critical image, its optical properties must be borne in mind, and it must be remembered that to obtain parallel light the condenser must be placed close to the flame of the lamp, and with its flat side against the flame. The bull's-eye must, of course, also be placed both centrally and at right angles with the direction of the light. It is an assistance to beginners if they do their focussing, both with bull's-eye and even with condenser, upon a sheet of note-paper placed in the requisite position. For parallel light the paper should be placed some feet away, and should then show a disc about the diameter of the bull's-eye, which does not vary much in size when the paper is shifted backwards

and forwards. The ordinary bull's-eye will only do this imperfectly. If the bull's-eye be used, it must be properly and carefully adjusted, or it will only interfere with the proper focussing of the condenser.

Corrections for Cover-Glass.—The disturbance set up by variations in thickness of cover-glass from that for which the objective has been corrected by the makers has been already alluded to (p. 135), and it has been explained that the necessary corrections can be made when the objective has a correction-collar by altering the position of the lenses, or, failing this, by altering the tube-length. As a matter of fact, a small readjustment of the lenses in the objective is equivalent to a large adjustment in tube-length, so that the latter can only be used to correct comparatively small variations in thickness of cover-glass, and it follows conversely that a slight variation in tube-length is not of much importance, at any rate with objectives of moderate apertures or magnifications. Mr. Conrad Beck has found by experiment that with a particular high-power dry lens an alteration of $\frac{1}{1000}$ inch in a cover-glass was equivalent to an alteration of no less than 17 inches in the length of the draw-tube.

All objectives of high aperture are more or less sensitive to variations in thickness of cover-glasses, but facility in the use of the correction-collar, in order to rectify the errors set up by the consequent under- or over-correction of the objective, needs some practice, and it really requires a trained and critical eye to enable these adjustments to be made satisfactorily. They may be made by observing the way in which the object comes in and out of focus. A minute speck in the object should be looked for, or some equally fine structure, such as a line or series of lines. The objective should

then be brought *slightly and in equal degree* both above and below the focus, the image being carefully watched meanwhile. If the adjustment is correct, the object will go out of focus in exactly the same manner whether up or down, seeming to diffuse or expand evenly on both sides; but if not correctly adjusted, it will form a diffuse ring or disc on one side, and fade away into mist on the other. By observing on which side the image remains or expands, and on which side it fades quickly into mist, the correct adjustments of the collar can be made to bring the image equal in behaviour on both sides. If the image seems to expand most when the object is outside the focus, there is spherical under-correction, which will necessitate opening the lenses—*i.e.*, moving the collar towards the zero mark. If the expansion is more evident when the object is within the focus, there is over-correction, and the lenses must be closed—*i.e.*, moved to the higher numbers. The marks on the collar generally correspond to the thickness of cover-glass in hundredths of a millimetre.

The tube-length must, of course, be adjusted for the length for which the objective is corrected, measured from the shoulder of the objective to the top of the tube at its eyepiece end, but allowing for nosepiece, etc.

The *Podura* scale, used by opticians, and especially recommended by Mr. Conrad Beck, is excellent to practise upon, and much easier than the Abbe test-plate, because if the objective is out of correction the appearance of the fine 'exclamation stop' markings upon the scale show a great difference when the lens is within or without the focus, instead of being identical. Not only is the definition bad, the marks running into each other, and not showing the central light marking in the middle of each dark 'stop,' but on one side we may

get a watered silk appearance, whilst on the other side we may get long streaky lines.

Correction by means of adjustment of tube-length is on similar lines, but has less latitude, and necessitates considerable variation in magnification.

The thickness of the cover-glass can be measured *in situ* by any microscope which possesses a sensitive fine adjustment of known pitch, with a graduated milled head. Dust or marks on the upper and lower surfaces of the cover-glass are successively focussed, and the respective readings of the fine adjustment noted. Then the difference in focus, multiplied by the refractive index of glass (1·5), gives the thickness of the cover-glass. If the graduations on the correction-collar of the objective correspond to thicknesses of cover-glass, it is only necessary to set the correction-collar accordingly, observing carefully the required tube-length. This method, however, is manifestly inferior to the former one, which is that adopted by all skilled observers.

As already stated, there is no uniformity in the thickness of cover-glass for which objectives are corrected by different makers, from 0·006 to 0·008 of an inch being common.

Care of Eyes.—There are a few more suggestions that may be useful to the beginner. The first is to remember *never to use a stronger light than is necessary*. Nothing is more fatiguing to the eyes, or more likely to work mischief, than excessive glare; but if reasonable precautions be taken the use of the microscope does not seem to be injurious to the eyesight. Some people are much troubled with what are called ' floating flies ' in the eye, but this is to a certain extent a question of ease of position. The second rule is to accustom oneself to keep *both* eyes open. The screwing up of the eye

not in use is a most injurious and unnecessary habit. At first, doubtless, some difficulty will be found, and the eye that is not looking down the tube will be distracted by external influences, but this difficulty is only temporary, and a little perseverance will overcome it. Some writers recommend a shade, but the author has advised many beginners to commence simply by holding the hand a short distance from the eye, and gradually to move it farther away as they gained experience, until finally it was no longer necessary at all. All workers have a tendency to use one eye more than another, and in this case the eye most used becomes generally rather less sensitive to brightness of image, but more capable of perceiving critical points. But every worker should learn to use either eye with equal facility.

Coloured Screens.—Various tints of blue or yellow glasses, or a disc of ground glass, are useful for moderating the light, and in some cases for accentuating the image (see p. 105). For visual work yellow light with a pale blue screen is most restful to the eye, but the eye is most sensitive to yellow-green, and the optician corrects his objectives for this accordingly. Deep blue is irritating to the retina, and difficult to see through, though the blue-violet end of the spectrum with its shorter wave-length has more resolving power. Daylight, though very comfortable, is too diffuse for critical work.

Remember to use no higher magnification than is absolutely necessary. The real microscopist uses the lowest power that will serve his purpose, for reasons that a very slight acquaintance with the microscope makes abundantly evident, and in all probability the most generally used lens is the modest inch.

Care of Fine Adjustment.—Remember also that the

fine adjustment is a delicate piece of mechanism, and endeavour to save it (and the mechanical stage, if there be one) from the very first. Any one of the microscopes described will satisfactorily and easily focus a $\frac{1}{6}$-inch by means of the coarse adjustment alone. Do not bear heavily upon any of the adjustments; endeavour to balance them gently between the finger and thumb, that the motion may be uniform; and do not on any account roll the fine adjustment by pressing one finger to one side only of the milled head.

The fingers can easily be trained to make minute movements of the slide and so to save the mechanical stage.

Care of Objectives.—Objectives should be carefully treated, and it should be borne in mind that they are delicate pieces of apparatus. Dew on a lens should be allowed to evaporate, dust on the back lens should be removed with a soft camel-hair or sable brush, and if the lenses really require cleaning, a specially soft piece of chamois leather, cambric, or silk should be kept free from dust, and used for that purpose only. The lenses should never be unscrewed—that is a matter for a first-rate optician only, and the maker is the proper man. An oil-immersion lens should be carefully and gently wiped immediately after use, and if by chance any of the oil should have dried on the lens, it is best to put another drop of oil on it, and to leave it for a time before wiping clean. Dr. Henri Van Heurck says there is nothing better than the ordinary saliva of the mouth for cleansing immersion lenses, but he worked on diatomaceæ, and not in a bacteriological laboratory ! If benzole or xylol are necessary, they should be applied to a piece of blotting-paper, and not allowed to soak into the mounting of the lens. Under any circum-

stances use as little pressure and friction in cleaning as possible. It sounds incredible, but the writer has seen a student try to clean the front lens of an objective by scrubbing it briskly on the bench at which he was working. When objectives are in use, but temporarily removed from the microscope, they should be laid end upwards on the table to keep out the dust. For this reason it is well also to keep one of the eyepieces habitually in the tube of the microscope.

A glass shade is preferable to the ordinary wooden case, except, of course, for travelling, as the microscope is apt to get jarred or knocked about through being constantly taken out of and put into its case. It is well, however, to remember that a microscope should not be allowed to stand in direct sunlight, if for no other reason than that the heat might prove injurious to the balsam or cement connecting the lenses of the objectives.

Care of Oculars.—The eyepieces, like the objectives, should be kept clean and free from dust. The dust can readily be localized by reducing the light by means of the iris diaphragm or otherwise, and then rotating the eyepiece. The objectives should not give much trouble in this way, and it will often be found that what was apparently dust on the back lens of the objective was actually dust on the cover-glass of the object or on the top lens of the condenser. This can be localized in like manner by moving the slide or rotating the condenser.

Use of Camera Lucida.—Some little practice in using any form of camera lucida, as described in Chapter V., is always necessary. The real secret of success lies in arranging the illumination satisfactorily, and this is more easily arrived at, whether the camera be fitted with moderating glasses or not, by using two lamps, one for the microscope as usual, and one to illuminate the

paper. A little adjustment of the light from each lamp is necessary until the pencil and drawing can be seen without losing the image in the microscope.

Beale's camera lucida is perhaps the most popular. A makeshift camera on this principle can be made by means of a piece of cork, a cover-glass, and a couple of pins, all that is necessary being to adjust the cover-glass in front of, but at an angle of 45 degrees with, the eye-lens of the eyepiece. The cover-glass gives, however, a somewhat troublesome double reflection, and this is obviated in Beale's arrangement, as supplied by the opticians, by the use of tinted glass. The Abbe form of camera lucida is considered by many workers to be the best in the market, but it is also the most costly. The paper must be parallel to the object, and therefore if the microscope is to be used in any but the vertical position, the drawing-board must be sloped accordingly. The mirror of the camera lucida must also be set at an angle of 45 degrees, or the drawing-board sloped proportionately.

In the newer form of combined eyepiece and camera lucida, made by various makers, some little practice is required to get the pupil of the eye placed in such a position over the prism that neither the image of the object nor of the paper overpowers the other. The beginner will find that in all forms of cameræ lucidæ the secret of success, as we have already pointed out, lies in the proper adjustment of the illumination for both microscope and paper. It is here that the value of an independent lamp for the paper or tinted glasses in the camera lucida makes itself felt. With low powers the illumination in the microscope is the stronger, and the lamp flame must be adjusted accordingly, or a piece of white paper even may be placed

over the mirror when that is used. With high powers the paper is generally the brighter, and tinted screens must be used or the light modified. The usual standard for distance between eyepiece and table is 10 inches, and this should be adhered to approximately. Any variation will alter the size of the drawing. It may not be superfluous to repeat that short-sighted people will require to use their spectacles if they are to see the paper and pencil clearly. The pencil should have a sharp point, and the lines should not be drawn too heavily in the first place. With all forms of drawing apparatus the paper must lie in the position for which the camera lucida is designed, as detailed above, or the result will be an elliptical image. Convenient adjustable drawing-boards are supplied by Baker, Watson, and other makers.

Use of Micrometer.—The use of the stage micrometer in connection with the camera lucida will suggest itself to anyone. It is only necessary to replace the object on the stage by the micrometer, taking care not to alter the other adjustments of the microscope, and to note the measurements thus shown upon the drawing. Supposing the portion of the drawing to be measured corresponded with $\frac{1}{100}$ inch as shown on the stage micrometer, and with 1 inch when measured with an ordinary rule, the actual magnification on the drawing is 100 diameters. If the micrometer has a millimetre scale it will be necessary to provide oneself with a rule divided in millimetres, or to convert the English measurements accordingly, either by reference to a table or by calculation. For rough purposes the English inch may be taken as 25·4 millimetres (see scale in Appendix).

The use of the stage micrometer in conjunction with

the eyepiece micrometer has been dealt with on p. 111. In making measurements by this method when using high powers difficulty is often encountered in causing the object on the stage or the stage micrometer to come into exact alignment with the lines in the eyepiece. To obviate this a mechanical stage is a great convenience, or the form of eyepiece micrometer designed by Mr. Jackson, with a slight adjustment to the scale by means of a screw. The most perfect form of micrometer eyepiece is the screw micrometer, containing one fixed and one travelling wire, the movement of the latter being accurately recorded by means of a drum, whilst each revolution of the drum corresponds to one of many serrated teeth in the field of view (Fig. 74). It is important to note whether the two wires exactly coincide when the drum is at zero; if not, they must be adjusted by loosening the drum-screw and re-setting the drum.

Measuring.—When the student is making accurate micrometric measurements it should be remembered that the divisions on the stage micrometer are not uniform, and that the mean value should accordingly be taken. Further, the stage micrometer should be used in every set of measurements, the usually recommended plan of making a record with different objectives and eyepieces once for all, for comparison, being manifestly untrustworthy. Errors due to diffraction in the real edges of objects mainly affect high powers, and it may suffice here simply to mention their existence. An example of some high-power measurements taken by the writer with a $\frac{1}{8}$-inch immersion objective and a screw micrometer eyepiece may be of service. Each of ten $\frac{1}{100}$ of a millimetre in the stage micrometer was taken, the whole added up, and divided by ten, giving a value of 2·43 revolutions of the micrometer eyepiece

for each $\frac{1}{100}$ millimetre. The stage micrometer was then removed and the object measured *without alteration of objective or tube-length*, and found to be 3·2 of a revolution. Accordingly $2{\cdot}43 : 3{\cdot}2 :: 0{\cdot}01 : x$; therefore $x = \frac{3{\cdot}2 \times 0{\cdot}01}{2{\cdot}43} = 13{\cdot}168\ \mu$ = the size of object required. Or, put in another way, if $\frac{1}{100}$ millimetre = 2·43 revolutions, 1 revolution $= \frac{0{\cdot}01}{2{\cdot}43} = 0{\cdot}004115$ millimetre $= 4{\cdot}115\ \mu$. Therefore the size of the object is $4{\cdot}115 \times 3{\cdot}2 = 13{\cdot}168\ \mu$. Of course, accuracy beyond $0{\cdot}25\ \mu$ is not to be expected, even by the most skilled observer, as readings to $\frac{1}{100}$ of a revolution are not possible with such high powers, and so near the limits of resolution, therefore the size of the object may be put down as $13{\cdot}25\ \mu$ (to convert to inches see Appendix). With such high magnifications it is also difficult sometimes to know which is the exact edge of an object, and it may be necessary to measure from the inner edge of one diffraction band to the corresponding inner edge of another, using the largest apertured objective and open iris diaphragm; whilst the lines of the micrometer, fine though they are, may still be too coarse, and must be mentally bisected—an alternative way to taking corresponding edges. When the object is very small the micrometer wires may need to be set beside it, and an ocular comparison made, or the object may be quite hidden. A mechanical stage is of great assistance in bringing the edge of the object into alignment with the fixed wire.

Test Objects.—In concluding these hints on the actual management of the microscope, the beginner is strongly advised to obtain a few ' test slides,' and diligently to practise himself in their examination. For low powers there is nothing more suitable than the fine hairs on

the tip of the proboscis of the blow-fly. These should come out quite sharp, black, and finely pointed. For medium powers the old-fashioned 'podura scale' (*Lepidocyrtis curvicollis*), mounted dry, may be recommended, and if the beginner can get the 'hairs' on these sharp and well marked, having the appearance of minute exclamation stops, and not running in the least into each other—we say nothing of interior 'marks'—he has learnt much in the management of his microscope, and in obtaining a 'critical image.' Unfortunately, the scales themselves vary very much, and it is not always easy to pick out a coarse enough scale that will be within the beginner's or even the objective's power. In obtaining such a critical image the worker will bear in mind not only what has already been said on adjustment of the light by means of the condenser and otherwise, but also the warning against an excessive shutting down of the cone of illumination by means of the iris diaphragm. This latter, whilst apparently at first sight increasing the contrast, really breaks down the definition of the objective, and leads to woolliness of outline in each individual mark on the scale, or even to a ring of refracted light round each. For high powers, especially immersion lenses, a slide of bacteria is suitable—say a slide of *Bacillus tuberculosis*, showing the characteristic beaded appearance. The diatom *Pleurosigma angulatum* is also a useful test object. To test for colour a thin section of deal may be used, or preferably a hair of *Polyxenus lagurus* or the diatom *Pleurosigma formosum*. For flatness of field a stage micrometer is better than an Echinus spine. These are mentioned as helpful aids in mastering the use of the microscope only. The proper testing of objectives requires long practice, skill, and experience.

The **Abbe test-plate** requires so much practice, and is so misleading in unfamiliar hands, that it is merely mentioned here. Full instructions are supplied with it by the makers—Messrs. Zeiss.

Adjustments.—If the microscope requires adjustment, these adjustments should be made with the utmost care. Most microscopes by our best English makers have the wearing parts 'sprung' so that the adjustments may be readily effected, but even then a little attention to the tools with which the work is done may be recommended. The screw-driver, for instance, should be in good condition and fit the screw.

The body-tube should not be removed from its bearings without careful marks being made to insure the re-engagement of the same teeth when it is replaced. For lubrication, only the best watchmaker's oil should be used, and used sparingly. When travelling, careful packing with soft tissue-paper is necessary, or parts and screws are apt to shake loose.

It is well also to bear in mind that the lacquer on the brasswork of the microscope, placed there not so much for appearance as for the prevention of oxidization, is destroyed by alcohol.

Finally, our advice to the beginner who may wish to oblige a friend by lending him his microscope is—don't.

CHAPTER VIII

PHOTO-MICROGRAPHY

THE first question generally asked is whether a regular photographic camera is necessary, and to this the answer must be in the affirmative. The main reason for this is that absolute rigidity with absence of movement is a *sine qua non*. An ordinary photographic camera could doubtless be fitted to an efficient base board by anyone with a mechanical turn, or who is used to carpentry; but the adjustments would be less satisfactory than in a camera made and designed for the purpose, whilst the cost of making such a baseboard and adding the necessary fittings would go some way towards the cost of a proper camera. A further reason is that the ordinary camera has insufficient bellows extension. A modern photo-micrographic camera extends to at least 30 inches, and often to much more, in order to give as large a range of magnification and size of field as possible. Of course, photographs have been taken with an ordinary camera, supported at the necessary height by books of the requisite thickness, and with the lens removed; but though the results seem to please their authors, they would scarcely bear much criticism.

The miniature cameras made for attachment to the body-tube of the microscope, without further support, can give small photographs with moderate powers, but can scarcely be recommended for serious work.

Great stress has been laid on absence of vibration in the supports or surroundings of photo-micrographic

cameras, but the author's experience has been that this is overestimated, provided the table or supports are firm and rigid. Of course, a ground floor is preferable to an upper floor, but street traffic outside or doors banging inside are disadvantageous even in a cellar.

The essentials of the microscope used in photomicrography are that it should have a first-rate fine adjustment and a good sub-stage condenser, whilst a rigid tripod stand, a wide body-tube, and a mechanical stage are all advantageous.

Horizontal and Vertical Cameras.—The next question relates to the respective advantages of a horizontal and a vertical camera. Dr. Henri Van Heurck, the well-known diatomist, did nearly all his work with a vertical camera, and there is much to be said in its favour (Fig. 109). It is convenient to use. The microscope is in the upright position, to which the observer is accustomed, and the adjustments of the microscope are thus more familiar to him, especially because he continues to use the mirror to reflect the light. It is easy to draw the camera bellows up out of the way, and to make most of the adjustments in the ordinary manner, and then to make the final connections; whilst, if the camera be of the box type, with a large door through which the head can be put to look down the microscope-tube, the matter is simpler still, though in this case there is no adjustment of camera length. Moreover, there are certain cases, such as those in which the object is but temporarily mounted—say in water, or otherwise—or in which comparatively large objects, such as embryos, are to be photographed in liquid with very low powers, where the camera *must* be used in an upright position. But there can be little doubt that the horizontal camera (Fig. 110) is more satisfactory for all-round work, and,

as a result, it is generally used and preferred. Its solid base gives complete rigidity with every facility for long extension, and it lends itself in particular to the adjustment of illuminant and microscope, with their inter-

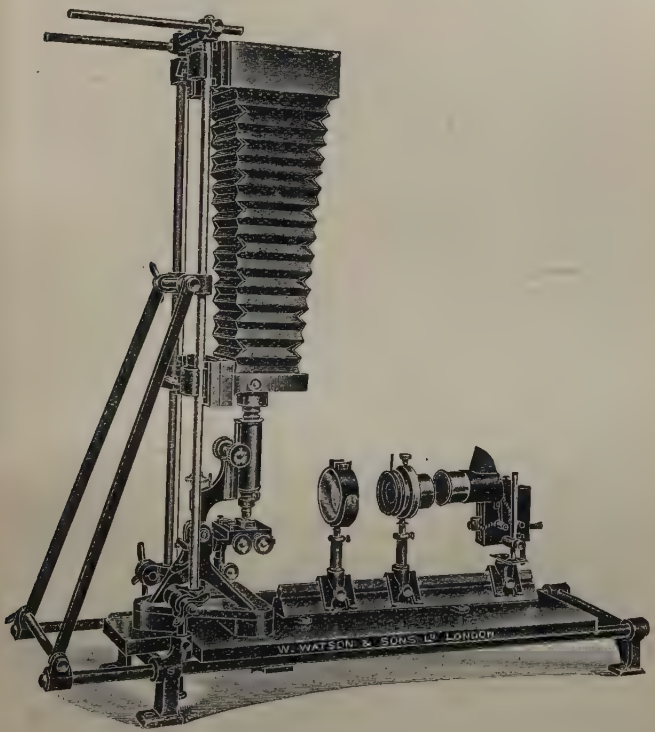


FIG. 109.—DUPLEX VERTICAL CAMERA.

vening accessories, and more especially to the more recent arrangement of optical bench, which has done so much to simplify and co-ordinate the necessary optical adjustments.

Choice of Camera and Stand.—It is not proposed to speak here of the most elaborate forms of photo-micrographic cameras, with all their various methods of interchange for photo-micrography, for projection, for lantern work, for reflected projection, etc. They are as costly as they are elaborate, and are mainly suitable for institutions and lecture-rooms. For ordinary workers a much simpler and less expensive camera is sufficient, and is capable of quite as efficient work. The following are its essentials: It must have a solid and steady base, and nicely-turned legs are not required. The part of the base which supports the microscope must be long enough to hold not only the microscope, but the lamp or other illuminant, and there must be room between the two for condenser, cooling-trough, and stand for coloured screens. All these should be arranged on stands running between parallel guides, so as to maintain their alignment with the optic axis of the microscope, whether they are brought forward or pushed back. The stands which support each piece of apparatus should, for the same reason, be square, and not round, and each piece of apparatus must be capable of adjustment to a small extent vertically and horizontally. A camera of this sort (without parallel guides) is illustrated in Fig. 111, the base-board swinging round on a pivot to facilitate visual adjustment.

Optical Bench.—The advantages of the optical bench are apparent. In its simplest form this is merely a heavy triangular iron bar, carefully planed, screwed to the base-board of the camera, and carrying on its edge the light and optical accessories on saddle-shaped bases. As has been already stated, this simplifies matters marvellously *once the primary adjustments are made*, but a couple of parallel wooden guides make a good, if less convenient, substitute.

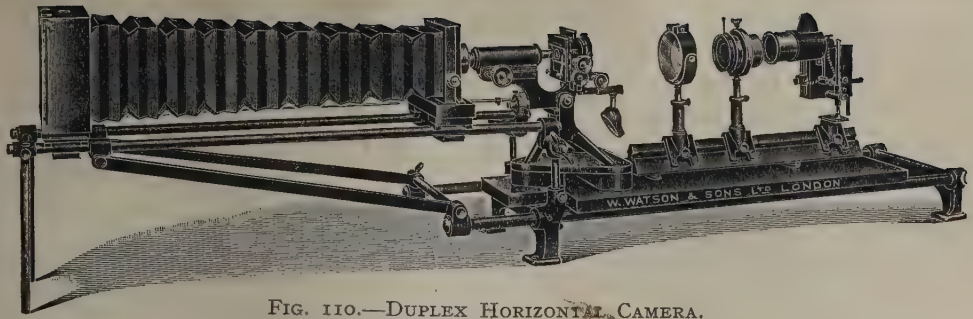


FIG. 110.—DUPLEX HORIZONTAL CAMERA.

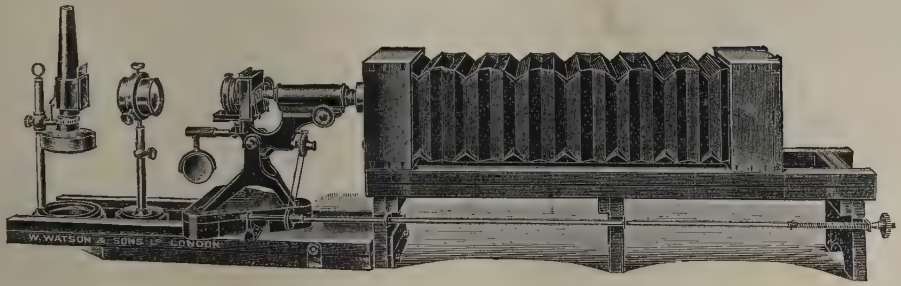


FIG. 111.—STUDENT'S CAMERA.

Fig. 112 represents one of the best forms of optical bench known to the author, made by Watson, though a somewhat similar form with shorter camera is made also by Swift. It is simple, absolutely rigid, most efficient, and not too expensive. The light is enclosed in a Russian iron case with side-door, and the optical bench is fitted with condenser with iris diaphragm (concerning which more will be said presently), cooling-trough, and auxiliary iris diaphragm with removable illuminator for low-power work.

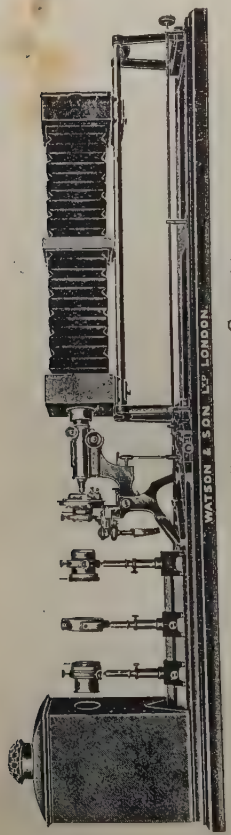


Fig. 112.—Laboratory Camera.

Figs. 109 and 110 represent a combined horizontal and vertical camera, and the ability to use the camera in either the horizontal or the vertical position is a distinct advantage, the two adjustable stays giving a degree of rigidity which is generally somewhat lacking in instruments of this type. The microscope is fastened to a table of the necessary height.

The camera itself is raised above the base-board, so as to bring its centre level with the optic axis of the microscope. It should extend to at least 30 inches, whilst many extend to much more. The fittings of the camera will be described in due course, but it may be

mentioned here that the light-tight joint need be nothing more elaborate than a loose sleeve of black velvet attached to the camera front, and slipped over the microscope-tube, and held in place there by a rubber or elastic band. But a deep ring, blackened inside, fitted over the end of the microscope, and slipping over a similar ring at the end of the camera, but without touching, is more convenient, and absolutely light-tight. A shutter should, if possible, be provided. Beck makes a handy little nosepiece shutter.

The camera and base-board, if the latter are of wood, must be made of well-seasoned mahogany, and the camera must run smoothly and truly in its slides, whilst the focussing-screens and dark-slides must also go into position easily. The dark-slides, etc., should be constructed to take half-plate negatives, and should have carriers to take quarter-plate ($3\frac{1}{4}$ by $4\frac{3}{4}$ inches), and lantern-sized plates ($3\frac{1}{4}$ inches square) as well (the quarter-plate being the most generally useful); but it is imperative that the plates, whether used with the carriers or not, as well as the focussing-screens, should all lie in exactly the same plane, and therefore be at exactly the same distance from the microscope. The carriers, in particular, unless carefully adjusted, often cause trouble in this respect. Cameras of Continental manufacture nearly always require carriers to take English-sized plates.

Cameras and stands more or less approximating to the types described are made by all the leading English makers, and by Leitz and Zeiss on the Continent, and optical benches are fitted by Baker, Beck, Swift, Watson, and Zeiss. The convenience of the latter cannot be too strongly insisted upon.

Illumination.—Let us now deal with the illuminant. Lamp light—the light of an ordinary ½-inch paraffin microscope lamp—is quite satisfactory as far as it goes, but it needs a relatively long exposure, and for very high-power work with dark screens it is, unfortunately, not powerful enough. An inch lamp is somewhat better. The incandescent gas burner, which Continental makers are so fond of, is not satisfactory. It gives a good light, but spreads it over too large a surface, and if it be focussed to give critical illumination it gives an image of the texture of the mantle, which is fatal. Acetylene is really excellent. The light is good, it is small, it can be focussed, and it has high actinic properties; but it is sometimes unpleasant on account of its smell, and often troublesome. The ordinary incandescent electric lamp is the most unsatisfactory of all, because the filament is too obtrusive when the light is focussed. The Nernst lamp is an improvement on this, but its disadvantages have been dealt with on p. 117, and it is at present unobtainable. The 'Pointolite' is better. The arc lamp gives brilliant illumination of great actinic value, and a central spot of light which can readily be centred and focussed; but it is apt to decentre slightly at critical junctures and thus to give an unevenly illuminated field. Moreover, it generally needs a definite voltage and an ampèrage beyond the capacity of most electric-light wiring for domestic purposes. Excellent little hand-fed arc lamps can now be obtained (Fig. 83) which require a voltage of 50 or 60, and take not more than 4 ampères, and we have found them of no little service. The carbons are small in diameter, and being set at right angles minimize the possibilities of decentring. They can be had with either hand or clockwork feed, and work well on alternating or

direct current. A resistance will probably be necessary (see p. 11).

The heliostat is too uncertain in this country, and can, of course, only be used by day. For all-round work outside laboratories the oxy-hydrogen jet is an efficient form of illuminant. It is powerful, gives exposures only second to the electric arc light in shortness, and is capable of giving critical illumination. It is a favourite with many expert photo-micrographers. The jet may be a ' mixed jet '—*i.e.*, supplied with both hydrogen and oxygen under pressure—or it may be a ' blow-through ' jet, in which oxygen is used under pressure, and gas from the town mains. The latter jet is quite strong enough for most purposes, easier to manage than the former, and cheaper. There are several good jets on the market, of which Mr. Pringle's, with ' cut-off,' is the best. It cuts off the oxygen, except when the photograph is being taken, leaving a small jet of hydrogen or coal-gas burning, but without altering the adjustments, so that the light can promptly be turned fully on again. A cylinder for the oxygen is required, a pressure-gauge and regulator, and the necessary connecting tubing for oxygen and ordinary gas. Hard limes are best, and as these are apt to crumble after exposure to the air, the author can recommend some special hard limes, which he believes contain also magnesium, supplied by Townson and Mercer, of Bishopsgate Street Within, London, which are very durable, and give little less light.

Under any circumstances the lamp or other source of illumination must be provided with some means of adjusting it both vertically and laterally. This is nearly always provided with the oxy-hydrogen jet, and the ordinary microscope oil-lamp is capable of vertical movement. The horizontal adjustments are easily ob-

tained by arranging a wooden stand to run lengthways in the parallel guides of the base-board, and another stand above this running at right angles in similar parallel guides or between two screws in slots. The two can be clamped together with a thumbscrew. Such an arrangement could easily be made at home.

It will be found a great convenience if a similar stand is made for the microscope, but the more general method is to arrange a clamp of some sort and shoulders against which the foot may come, so that, once the microscope has been definitely adjusted in its proper position, and the shoulders fixed accordingly, it may be easy at any time to replace it and to clamp it securely, with the certainty that it is correctly placed. The Continental stand is so unsteady in the horizontal position that such a clamp is imperative; but the English tripod stand is nearly as steady when horizontal as when upright, and in the case of one of the author's own microscopes he has merely to lift the instrument from the table where he has been using it, incline the body horizontally, and drop the three feet of the stand into three little metal rings screwed on to the camera base-board, which were carefully placed correctly once for all.

Opaque objects must, of course, be illuminated from above or from the side, and any of the reflectors mentioned on p. 181 are useful; but most have a tendency to give one-sided illumination, with corresponding shadows. Two lamps are often necessary, and even then the exposure is relatively long.

Objects illuminated by the ' dark-ground ' method are apt to be ' fuzzy,' and require considerable exposure; whilst doubly refracting substances, such as crystals, horn, etc., used with analyser and polarizer, require abnormally long exposures.

Auxiliary Condenser.—The loss of light in photo-micrography being considerable, some means of strengthening it is generally necessary, and this is usually provided by means of an auxiliary condenser placed between the light and the sub-stage condenser of the microscope. A further necessity for such an auxiliary condenser is due to the importance in photo-micrography of illuminating equally the whole of the field. With ordinary visual work this is not necessary, and the advanced microscopist rigidly focusses the edge of his lamp-flame in the centre of the field (see p. 174) to get critical illumination here, and cheerfully neglects the comparatively badly illuminated portions of the field on each side. But this is manifestly not admissible in a photo-micrograph, and any alteration in the focus of the sub-stage condenser to do away with this light-streak would not only depreciate the image, but cause considerable loss of light. Accordingly the auxiliary condenser is interposed.

Adjustment of Auxiliary Condenser.—There are three ways in which this condenser may be adjusted, and this point is generally insufficiently dealt with in books on photo-micrography. To begin with, the auxiliary condenser can be adjusted to give convergent light or parallel light. Of these, the first is the one generally used, the bull's-eye being so adjusted as to bring the light to a focus 10 inches or so away from the sub-stage condenser, which is then focussed on this point. A little experimenting with the bull's-eye in various positions will be found very helpful and instructive. The plane side of the bull's-eye should be turned towards the light and brought comparatively close to it, as in this position the aberrations of the ordinary uncorrected plano-convex bull's-eye are least in evidence.

The room should be darkened, and a piece of white paper held in the beam of light coming from the bull's-eye. By moving this backwards and forwards an approximate position can be found where the light comes to a focus, and by slightly moving the bull's-eye itself this focus will be found to lengthen and shorten inversely as the corresponding conjugate focus from the lamp-flame lengthens and shortens. When the bull's-eye is adjusted to give parallel light, the disc of light upon the paper should be of the same size as the bull's-eye, and should remain of the same size, neither more nor less, whether the paper be brought towards the illuminant or receded, and when looked through directly the bull's-eye should be full of light at all distances. Unfortunately, the ordinary bull's-eye has so many aberrations inherent to its simple form of construction that only approximately parallel light and only approximately focussed light can be obtained by its means. The so-called ' aplanatic ' bull's-eyes are only a degree better, and it is here that the advantage of a really well-corrected auxiliary condenser becomes evident. Watson supplies such a condenser, designed by Mr. Conrady, which leaves nothing to be desired, and greatly facilitates good results, giving really critical light and, incidentally, considerably shortening exposures. A word may be said concerning the large condensers, 4 inches or so in diameter, found on some Continental photomicrographic stands. Of course, they take in a large quantity of light, but they take in more than the objective can utilize, whilst their spherical aberration renders the greater portion of the light transmitted by such condensers quite useless for practical purposes. Thus the small but really aplanatic condenser yields a brighter illumination, because losses by absorption and reflection

are reduced to a minimum, whilst those due to spherical aberration are entirely done away with. In fact, the clear diameter ($2\frac{1}{2}$ inches) of even this condenser is too large for most purposes, and an iris diaphragm is provided close to the lens so that its aperture may be reduced to any desired extent.

The second method of adjusting the condenser is to throw parallel, or approximately parallel, light upon the sub-stage condenser direct, and with an ordinary bull's-eye this gives as good, or even better, results than the method just mentioned of focussing 10 inches behind the condenser. Of course, were the light really parallel the sub-stage condenser would bring it to a focus upon the object once more; but this does not happen in practice (except with the well-corrected Conrady condenser), and if the sub-stage condenser is properly centred the field is filled with light and very evenly illuminated. Parallel light is mostly used with low powers, and the sub-stage condenser often removed.

The third method is to project the light into the plane of the objective itself—say about the position of its front lens—and this also is a general practice with low powers, the sub-stage condenser being omitted, especially with those planar lenses of 3 inches or more focus, which are used for the lowest-power work. But some Continental workers use this method of illumination even in high-power work, probably because it is easier to get a more evenly illuminated field, centring screws being a rare luxury in the Continental sub-stage.

Centring and Focussing of Illumination and Condensers.—This is a most important adjustment in high-power and even medium-power work, and must be understood and carried out carefully if good work is to be done. It is here that the worker will feel the

advantage of those means of vertical and horizontal adjustment for each piece of apparatus mentioned previously, and especially of an optical bench.

First set up the microscope horizontally or vertically, as the case may be, so that the eyepiece end is connected with the camera, and make the light-tight connection. Place the illuminant 1 foot or more beyond the sub-stage, so as to shine through the tube, or reflect it by means of the mirror. Then screw in a low-power objective, and examine the disc of light upon the ground-glass screen of the camera. The axis of the microscope should point straight down the camera base-board towards the light, parallel with the sides; it must be truly horizontal, and the disc of light should appear exactly in the centre of the ground-glass screen. This centres the microscope. Now remove the objective and the ground-glass screen, and look straight along the tube, and then adjust the illuminant until it appears in the centre of the limited field of view. This is the preliminary adjustment. Next move the camera in its slides until there is room for the head to be placed between it and the microscope, in order to enable the eye to look down the tube. It is a convenience in this respect if the whole portion of the camera base which carries the microscope, lamp, etc., rotates on a pivot to one side, or if the camera itself can be swung aside as in the vertical type.

We have now got the microscope in the place it will occupy in photographing, and we have also got the source of illumination approximately centred, but have still to centre the sub-stage and auxiliary condensers. Remove the latter. First centre the sub-stage condenser. This is done in the usual way (see p. 176) by using an ordinary eyepiece and a fairly low-power objec-

tive—say 1 inch—closing the iris diaphragm as much as possible, and racking the body-tube up or the condenser down until the edges of the diaphragm appear in the field, when the small central disc of light is to be centred with the centring screws. Make sure that the edges of the iris diaphragm are really in focus, or one may be focussing on the central beam of light, which, being probably oblique, will alter in position with each variation of focus, and so give misleading results. Then open the diaphragm, and focus the objective upon some suitable slide placed upon the stage. Next focus the condenser, and bring the image of the illuminant into focus. It will probably be found to be not quite in the centre of the field, and must be brought central by moving the illuminant, and *not* the condenser. This is sometimes rather a troublesome matter, as very small adjustments are required, and the hands cannot always reach the light whilst the eye is at the microscope-tube, so that assistance from a second person is of service. The auxiliary condenser (which has been removed altogether for the time being) now alone remains to be centred, and where it is not provided with an iris diaphragm this can only be done by means of a cap of brass or blackened cardboard fitted on its anterior face and perforated with a very small hole. This small hole is focussed by adjusting the sub-stage condenser, but it is brought central by moving the auxiliary condenser vertically or horizontally, but not by altering the centring of the sub-stage condenser. Care must be taken that the auxiliary condenser is set true and square with the long optic axis, to which we have so carefully adjusted and centred everything—camera and microscope, sub-stage condenser, auxiliary condenser, and light. The auxiliary condenser may be centred before

the illuminant, but the other method is the easier in most cases. The illuminant may also be centred by projecting its image upon the closed iris diaphragm of the sub-stage condenser and looking at that from behind.

It is now necessary to decide at what distance from the lamp the auxiliary condenser shall be placed, whether to give parallel or convergent light as already explained, and if the latter, where the light shall come to a focus. The experiments with white paper will prove very useful here. The blackened disc is, of course, removed after the centring, just as the iris diaphragm of the sub-stage is re-opened. On examining the ground-glass screen, a bright and uniformly illuminated disc should be observed in the centre of the screen, and this is best judged by standing back some distance from the end of the camera, the room being darkened. Of course, with a 1-inch objective, most sub-stage condensers will not give a large enough field of light, and in this case the top lens of the sub-stage condenser can be removed, or the condenser can be removed altogether, and the light from the auxiliary condenser focussed on the objective so as to fill it fully. A piece of ground-glass interposed gives a very uniform field, but with some loss of light and definition.

The utmost care must be taken in observing whether this disc of light on the focussing-screen is really uniform. It is somewhat deceptive, and a slight readjustment may sometimes make all the difference; but if it is not uniform the photograph will show unequal illumination —that is, one side will be rather darker than the other, which is not satisfactory, though too often seen, especially with low powers. The sub-stage condenser, and even the auxiliary condenser, may need slight readjustment and re-focussing to obtain the best results, but

any great alteration will not be found satisfactory. A little patient experimenting, combined with clear comprehension of the optical principles involved, will be found to teach more than many pages of suggestions.

The advantages of the optical bench or of the parallel slides are now evident, and if there is any change of objective a slight readjustment *of the sub-stage condenser* to bring the light central once more is all that is required. It saves much labour if everything is left in place except the microscope, and the position of the latter is carefully marked, or slots made for its stand; but if the auxiliary condenser and illuminant must be removed, then some means should be adopted of so arranging or marking them that, when required, they can without difficulty be brought back exactly to their previous position.

Focussing the Image.—The preliminary focussing of all objects may be done upon the ground-glass screen in a darkened room; but this screen, however fine it may be, will be found too coarse for the final focussing of all but low powers. We must, therefore, have a further frame of plain glass fitting into the same slides, and once again it must be insisted upon that the inner surface of this must be in exact correspondence with the sensitive film of the photographic plate when the latter is in the dark-slide and in position in the camera. It is not a bad plan to fasten a thin cover-glass with Canada balsam in the very centre of the inner and rough side of the ground-glass screen. This makes a little transparent disc for examining the photograph, but it is scarcely big enough to be an efficient substitute for the complete plate-glass screen. In using the latter we have, of course, no surface to form an image upon, and we therefore need a focussing lens of some

sort. This may be of the simplest form, but its magnification should not be too high nor should its 'depth of focus' be too great. A dissecting lupe in a cardboard or wooden ring will serve, or one of the ordinary tripod magnifiers; but suitable lenses, with a screw ring for adjustment, can be bought from most opticians. An ink-mark is made on the inner surface of the plain-glass screen, the focussing lens is brought in contact with the other and outer side, and then adjusted so as to focus the ink-mark through the glass. It is now in focus for examining the image. Dr. Bousfield recommends the use of a double convex spectacle lens of about 8 inches focal length which may be mounted in a cardboard or other tube of suitable length, but the author has, personally, not found this of much service.

Final Adjustments.—The necessary centring adjustments have been carefully explained, and the image of the object on the stage may be assumed to be projected and focussed upon the ground-glass screen. It is advisable now to examine it carefully to see that it is correctly in the centre of the screen, and that its size corresponds with the plate to be used. It is well to mark the limits of a quarter-plate in pencil on the ground glass, whilst two lines drawn diagonally from corner to corner give the centre of the plate. Particular care should be taken at this stage to note if the illumination is even all over the field; if not, the sub-stage condenser may need a slight readjustment by means of its centring screws. It is also well to make now the final adjustments of the iris diaphragm belonging to the sub-stage condenser, bearing always in mind that the apparent sharpening of the image by closing the diaphragm is more apparent than real, and that any excess in reduction of aperture will give fuzzy diffraction effects in the photograph, as

well as prolong the exposure and increase any tendency to unequal illumination. This must always be remembered in dealing with thin slides or those with little contrast. Of course, the lower the numerical aperture of the objective, the smaller will be the cone of illumination that it will bear; but it is a fairly safe general principle to shut down the diaphragm cautiously and to stop as soon as, or very soon after, the image begins to show signs of reduction in brightness. But the diaphragm must not be left open too widely, or the definition will suffer and a poor photograph result. All these adjustments can only be carried out on the ground-glass screen. The plain-glass screen is then put in place of the ground glass, and the image is now invisible until the focussing lens (previously adjusted) is laid lightly upon the plain-glass screen; through this the eye looks directly towards the tube, the focussing lens performing as a positive ocular. The amount of light is now so great that it will be difficult to make any other adjustments than those for the final focussing, and for this purpose the lens is moved about on the glass whilst one hand adjusts the fine adjustment of the microscope. The excess of light may be modified by *temporarily* shutting down the iris diaphragm of the sub-stage condenser, or, preferably, of the auxiliary condenser.

An iris diaphragm on the auxiliary condenser is useful in modifying excessive light and in cutting off reflections from the inner surface of the microscope-tube, which sometimes give trouble, even though it is well blackened. A large body-tube is advantageous in this respect.

The extension of the camera gives considerable latitude in magnification independently of any change in objective or ocular, and, of course, with any change of

objective the sub-stage condenser will generally need re-centring. But unless the camera is fairly short it will be found impossible to keep the eye at the focussing lens and at the same time to reach the fine adjustment. It is, therefore, more than a convenience to have a light rod running along the camera with a milled head at one end and at the other a grooved pulley lying exactly in line with the milled head of the fine adjustment of the microscope, which is now nearly always grooved for this purpose, whilst between the two a thin endless cord passes. A counter pulley on the other side of the microscope saves unequal wear of the fine adjustment screw. The focussing adjustments can then be made with ease from the very end of a long camera extension. This arrangement is better than any Hooke's jointed rod.

Watson has, however, largely done away with the necessity for both ground- and plain-glass screens (as well as focussing rod) by inserting in their place a sheet of highly-glazed cardboard, which can be viewed directly from the inside by means of a small door cut in the side of the camera. The facilities this gives for ease of adjustment of all the centring movements is great, and when it is stated that the flagella of a bacillus like *Typhosus* can readily be focussed by this means, it will be seen that it is amply sufficient for most purposes. The author may perhaps mention that he uses this method almost exclusively. But of course a good condenser and a strong light, such as the arc light or the oxy-hydrogen jet, are necessary, and for this reason so much space has been given to the older and more general methods of focussing, etc.

Objectives and Oculars. — Formerly considerable trouble was caused by objectives having different visual and actinic foci, so that an image which was

perfectly focussed visually gave a photograph which was not in focus, and special photographic objectives were sold. But almost any of the recent achromatic objectives, particularly the semi-apochromats, will give excellent results, especially if used with screens or yellow sensitive plates. The apochromats, owing to their corrections for more than two colours of the spectrum, are best of all for photography, but, unfortunately, they are costly. They have a disadvantage in being somewhat round in the field, and they are not meant to be, and cannot be, used without compensating eyepieces. Achromatic lenses can be used with or without oculars, but the best results are obtained by using the special projection oculars mentioned on p. 84. These contain a diaphragm, which is adjustable to give its image upon the screen. They are marked with figures to facilitate reference, and should be re-focussed for any considerable alteration in camera length. They are generally sold marked $\times 2$ and $\times 4$, but usually give initial magnifications 50 per cent. higher. Ordinary Huyghenian oculars can be used with achromatic objectives, but perform only moderately, and high eyepiecing is a mistake in photo-micrography.

For very low magnifications—below 2-inch objectives—special aplanats are made by firms like Leitz, Reichert, Watson, and Zeiss, and these are specially constructed to be flat in the field up to the very margin. They may be screwed into a special fitment at the end of the camera, doing away with the microscope, unless the microscope-tube is made of specially large bore and with an extra wide screw in it to enable them to be used, as in Zeiss's photo-micrographic stand. The object is then generally supported on an independent

stand, which serves as stage, and the focussing is done by adjusting the stage rather than the lens or ground-glass.

It will readily be understood that any given magnification may be obtained by combining objective, ocular, and camera length. An inch objective and a ×6 ocular would give with the 10-inch tube a magnification of about 60 diameters visually, and the same in the camera when the screen is set 10 inches (to correspond to the visual distance) from the eye-lens of the ocular. If the camera be extended to 20 inches, the combined microscope magnification would be multiplied by 2 and become 120; if extended to 30 inches, multiplied by 3, and become 180, and so on. With the 6½-inch tube the initial magnification of the 1-inch objective being only 6½, the ×6 ocular would give a combined magnification with a 10-inch camera extension of 39, with a 20-inch extension of 78, and so on. Magnifications with other objectives can readily be calculated (see p. 142).

Cooling Troughs.—The heat of the illuminant, focussed as the latter is on a small space, may easily damage the object or melt the cement between the objective combinations. To obviate this a cooling trough is put between the two condensers. It should be of considerable size and have parallel sides. There is no better pattern than Kingsford's troughs, sold by Watson (see p. 124). Plain water is quite sufficient, but it should be boiled and cooled again to prevent air-bubbles appearing on the sides of the trough, and changed when it gets hot.

Plates and Screens.—Unless an object has good contrast, it is manifest that the resulting photograph will be lacking in contrast; but what is less evident is that

the stains with which microscopical slides are stained may differentiate structures sharply enough to the eye, and yet, owing to the varying actinicity of the colours (which are often also very deceptive in the real colour they allow to pass), may fail to differentiate the same structure satisfactorily in the photograph. An ordinary photographic plate shows great susceptibility to the rays at the violet and blue end of the spectrum, and very little to those at the yellow and red end.

If the object were merely black and white, this would be of less importance; but supposing we were trying to photograph a violet or blue object on a yellow ground, we should find that the object would impress itself upon the plate as fast as the ground, and there would be no contrast or differentiation. We should, therefore, need to keep back the violet of the object and to assist the yellow of the ground.

Our first method of dealing with this is to use an orthochromatic plate—namely, one made comparatively, but not actually, more sensitive to yellow; and our second method is to use, in this case, a screen of dense yellow glass, which will cut off, or at least keep back, the violet rays, and so assist the yellow rays to impress themselves upon the yellow sensitive plate. Of course, the treatment will be varied according to the staining, and it might even happen that with a pale yellow object we might need to use an ordinary plate and a blue or green screen. But enough has been said to show the nature of some of the difficulties besetting the photo-micrographer, and the way to deal with them. Generally speaking, the principle is to keep back the colour with which an object is stained by using a plate which is relatively less sensitive to that colour, and a screen (generally of an opposite colour) which tends to

nullify it. Usually specimens stained violet will require a yellow filter, purple and red a green filter, blue or green a red filter, and brown or yellow a blue filter.

The plate used in photo-micrography should therefore be an orthochromatic one. It should in nearly all cases be of *medium* rapidity, in order to get contrast and latitude of exposure, and it should be 'backed' to save 'halation' reflections. Ordinary plates can be treated at home with dyes to make them orthochromatic, but there are so many good plates on the market at moderate prices that it is scarcely worth the trouble. The 'Edwards,' 'Barnet,' and 'Ilford' plates are all well known. The first is perhaps the most popular, but according to some interesting tests made by the late Mr. E. J. Spitta, the 'Isochrom' plate of the Ilford Company has a wider range of sensitiveness to an extended spectrum, and is therefore superior. The 'Lumière' plate is rather more expensive, and the 'Cadett' plate has the largest range of all, but is awkward to develop in even the most subdued and 'safe' light, on account of this very sensitiveness, whilst it is rather slow, and does not readily give density. The plates recently brought out by Wratten and Wainwright are excellent. It is well to make a practice of using one kind of plate, and to adhere to it, except for some special reason. Plates, especially orthochromatic ones, should never be kept long, as they deteriorate rather quickly.

The necessary screens will be two yellow screens, one of them dense, two blue screens of the same description, and a signal-green or pot-green screen—the most useful of all, especially with achromatic lenses. Gifford's F-line screen (p. 106) is a very useful one, whether made of glass and gelatine or of signal green glass and malachite

green and glycerine, which latter passes more light and is more truly monochromatic. Wratten and Wainwright's screens are well known, but the new Chance glass screens are best of all. All these screens are dealt with on p. 106.

Exposure.—The question of exposure is difficult to give hints upon, as it varies enormously with the light, the plate, the magnification, the aperture of the objective, and the object itself, and trial and experience are necessary. Two things may be said, however; firstly, that orthochromatic plates of medium speed have unexpected latitude in respect of exposure; and secondly, that in case of over- or under-exposure, it is a fairly safe rule boldly to halve or double the exposure given, as indicated by the state of the plate on development, and not to try intermediate differences of time. An oil-lamp would naturally require very much longer exposures than lime-light. An inch objective with an oil-lamp, for instance, might require a minute's exposure, and a $\frac{1}{6}$-inch three or four minutes, where the oxy-hydrogen light might require ten seconds and forty-five seconds respectively.

Reds and yellows photograph very slowly, even on orthochromatic plates, and the use of yellow screens increases the exposure two or three times with orthochromatic plates, and very much more with ordinary plates; whilst a Gifford's green screen is about half as slow again as a yellow screen. Orthochromatic plates are, of course, much slower than ordinary plates, and backed plates slightly slower than unbacked ones.

Very dense objects also require a relatively long exposure, and very thin ones a short exposure, whilst it is often difficult to get contrast with the latter. Slides deeply stained with red and similar dyes require long

exposures, and often give trouble even with orthochromatic plates and suitable screens.

The higher the magnification, the longer the exposure, as will be seen by the examples given above, the amount of light passed varying as the square of the magnification. But high *aperture* apart from this shortens the exposure. Immersion lenses are quicker than high-powered dry ones.

The method of exposure itself is simple enough. Before exposing the slide it is well to take a last look at the focussing, to make sure that it has not altered, owing either to a slight slip in the microscope (a fault not uncommon when the camera is used vertically), or to expansion of the brass-work with the heat of the illuminant. The exposure-shutter is then closed, or a piece of black card put beneath the condenser or objective, the shutter of the dark-slide withdrawn, the exposure made, and the exposure-shutter and dark-slide closed again. Care must be taken in inserting the dark-slide or in opening its shutter not to shake the camera. The time should always be taken with a watch with a seconds-hand, and it is of much service for future work and reference if a careful and systematic record be kept of exposure, camera-length, objective, ocular, aperture of condenser, screen, plate, and illuminant, together with one or two particulars as to the staining or otherwise of the slide.

Development and Fixing of the Plate.—It will be assumed that the worker has some acquaintance with ordinary photography, and therefore little need be said on this point. Orthochromatic plates must be developed in as subdued a light as possible, and as they are specially sensitive to yellow light, the lamp will require a safe red screen, and even then should be turned

down low. The plate should be exposed to even this light as little as possible until it is, at any rate, partially fixed, and the developing dish should be covered with a screen of cardboard as much as possible.

Almost any developer may be used, such as can be obtained from any dealer in photographic requisites, and the worker is advised to use that one with which he is most familiar, and to endeavour to become thoroughly familiar with the management of one developer rather than to experiment blindly with several. It may be mentioned, however, that either pyrogallic acid, hydroquinone, or metol developers give excellent results, as does also 'Rodinal'; but the latter is apt to give a negative wanting in density, and pyrogallic acid is rather apt to fog. The requirements of a photo-micrographic negative are different from those of a landscape or portrait negative in that much contrast and as sharp blacks and whites as possible are needed. The author almost invariably uses metol-hydroquinone, and confesses to a weakness for using all his photo-micrographic chemicals in 'tabloid' form on account of their keeping properties, their reliability, their portability, and their ease of measurement. Such a developer as made by Burroughs, Wellcome and Co. is suitable for plates or films, bromide papers, or lantern-slides. Full particulars are given with the packets, and no formula need be given here, but two or three hints may be of service. With metol-hydroquinone the image comes up very rapidly at first, but if the plate is properly exposed, it should take density more deliberately, each detail coming up in order. There is little tendency to fog, and it is generally advisable to leave the development rather longer than would be advisable in ordinary photographic work. When complete, the whole plate should be quite black

all over, the image generally, but not always, showing through at the back, and always seen when held to the light. The whole process generally takes about five minutes. If the plate is under-exposed, the image comes up slowly, and looks thin when held to the light, or there are sharp contrasts without detail appearing properly; whilst if over-exposed, the image comes up quickly and darkens rapidly all over, forming a dense negative with obscured detail.

In case of over-development it is wise to have a bottle of potassium bromide (1 grain to a drachm of water) close at hand, and to add a few drops to the developer. This slows the development and increases the contrast and density.

It is not advisable to use the developer for more than three or four plates, and even then it will be found to slow each time it is used. The plate should always be lightly brushed with a soft copying-press brush when taken from the slide, to prevent pin-holes, and the backing washed off with a damp sponge, keeping the film itself dry. The developer must be poured over the film with an even sweep, so that the whole of the film is wet simultaneously, covered at once with the cardboard cover, and gently rocked in the usual way. It should be examined as little as possible, especially towards the beginning, but none the less requires careful watching.

Fixation.—This is done in the usual way with hypo-sulphite of soda. After removal from the developer the negative is washed under a rose for a few moments, and then put into the hypo. It is convenient to make a saturated solution of the latter, and to dilute it with about half its volume of water, as too concentrated a solution prolongs the fixation greatly. The fixation

should be continued for some little time after the silver has apparently left the back of the plate, as more plates are spoiled by cutting this process short than in any other way. After fixation the plate is washed for a couple of hours or more in running water, or for an hour under a rose, and then dried in a rack. It is advisable to wipe the back of the plates with a cloth, not to have them too close together when drying, and to support them so that they drain to one corner.

Reducing and Intensifying.—For reducing, the author is accustomed to use 'tabloid' ammonium persulphate or potassium ferricyanide, the former for under-exposed negatives, where contrast is too high but the details require little interference, and the latter for over-exposed and dense negatives, where the details are confused and require clearing up. Full details are given with each packet. For intensifying, 'tabloid' mercuric iodide and sodium sulphite gives good results. It must be confessed, however, that in photo-micrographic work the processes of intensifying or reducing a negative often fail to give satisfactory results, and under any circumstances require considerable experience.

Printing.—Silver prints are seldom quite satisfactory with photo-micrographs, and a gas-light paper is so much better and more convenient that it is better to use it always. Like many other photographers, the author has found Eastman's 'Nikko' paper admirable for this purpose. It gives good blacks and whites, and is easily controlled. The glossy surface is always chosen, and squeezing on to a ferrotype plate increases this surface. The exposure is done by direct contact with the negative in an ordinary frame, the frame being held about 18 inches from a gas lamp or incandescent electric

lamp, and moved slightly in a circle to get uniform lighting. It is a good plan to mark the distance on the wall or elsewhere. A negative of ordinary density generally requires about ten to fifteen seconds' exposure, a dense one twice as long. A very suitable developer with which to get good black tones is metol-hydroquinone, used rather strong, or 'Rodinal,' though the makers recommend 'Amidol.' With both, the paper should be washed for a minute in water before adding the developer, and the development should be watched, and stopped slightly before completion, as it continues for a little time after the print is removed from the solution. The image appears almost at once, and the whole is completed in three or four minutes. The remaining processes of washing in several changes of water, fixation with hypo, and of final washing, are similar to those of negatives. Under-exposure gives prints wanting in contrast and in black and white, whilst over-exposure gives harsh, staring contrast, without detail.

Lantern-Slides.—The best results are given by the wet collodion process, but this is not easy for the ordinary worker to carry out, and Edwards' 'Kristal' plates can be recommended for the purpose. They are printed direct from the negative by artificial light, very much as with bromide papers, but require longer exposures, and can be developed with metol-hydroquinone in the usual way, by shaded artificial light, without screens. Full particulars are supplied with the plates, and black prints with very clear background must be aimed at. With good negatives, good results are easy to obtain. The binding up with plain glass and the interposition of suitable masks offer no difficulty. Enlarging, whether of negatives, positives, or of lantern-plates, scarcely comes within the scope of this book.

CHAPTER IX

MICROSCOPICAL TECHNIQUE

(*a*) Preparation and Mounting.

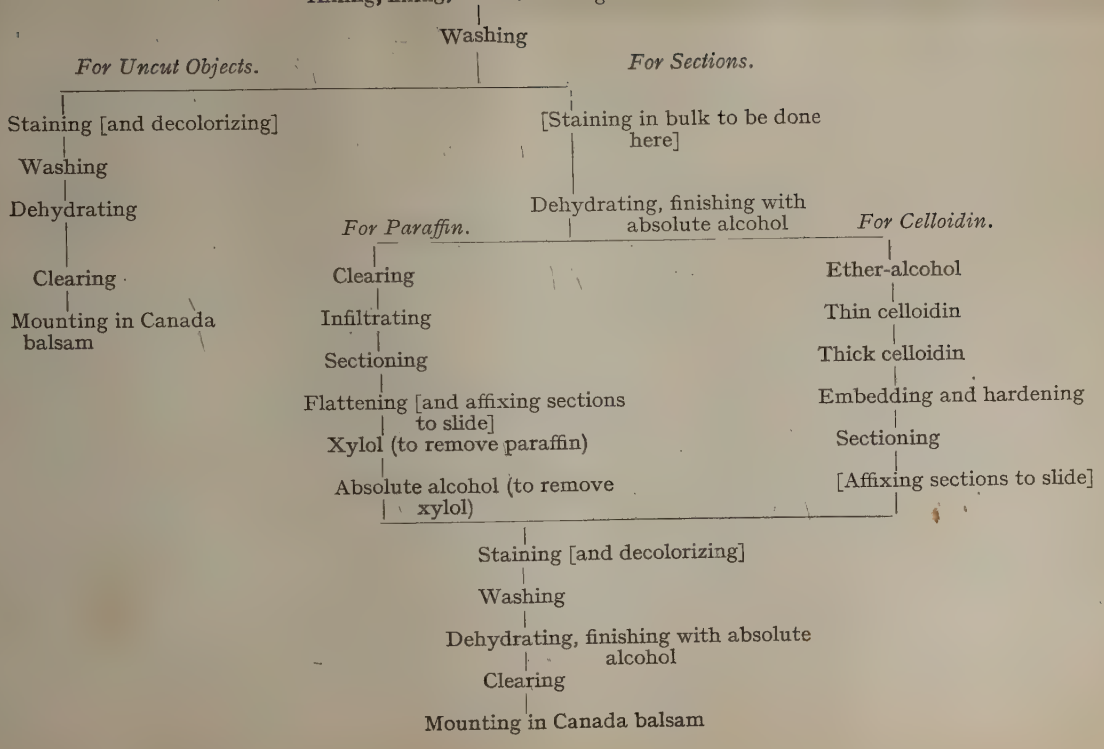
THE preparation of objects for the microscope is now almost a science in itself, and one upon which depends much of the knowledge which we can add to our own or to the common stock by means of that wonderful instrument of precision, the modern microscope.

It is, of course, only with low powers, and when the nature of the investigation admits of it, that an absolutely unprepared and unarranged object can be examined. For this purpose a pocket lens is infinitely preferable to the compound microscope, with all its complications and refinements. For examination with the latter instrument even opaque objects need to be properly displayed; whilst objects to be examined with transmitted or direct light—that is, by means of light which passes through the object—require very careful preparation beforehand.

Just as the previous chapters have endeavoured to give practical instruction in the use of the microscope and its accessories, leaving its applications to be dealt with in books specially devoted to such purposes, so the present chapter deals with the broad general principles underlying various methods of microscopical technique, and leaves their application to be found elsewhere. Special endeavour has been made to give assistance in those practical details, for instance, in section-cutting, which the specialized text-books generally omit.

General Principles.—It will be well, before going further, to explain to the uninitiated the principles of microscopical technique and their order. The first procedure is **fixation,** by which is meant not only *killing* an animal or plant, yet leaving its form as it was in life, but *fixing* its tissues in such a way that they may undergo the remaining processes well, not merely with a minimum of shrinkage, distortion, or disintegration, but so that the stains used may work satisfactorily. This process of fixation is of the utmost importance, and in most cases the final results largely depend upon its being carried out properly and thoroughly. The fixing agent is then removed by washing, and the object *stained* at once direct from water, if it is to be mounted in an aqueous medium such as glycerine; but if it is to be mounted in a resinous medium like Canada balsam, all the water must be carefully extracted by means of alcohol, which has a great affinity for water. This is known as **dehydration.** The *staining* may come in either before or after dehydration, according to the nature of the stain decided upon. Then the alcohol is itself removed, and the section made clear and transparent by means of a **clearing** agent. If *sections* are required to be cut, the tissues must be supported by embedding, freezing, or infiltrating with paraffin or celloidin. Sections having been cut, their *staining* follows (unless the tissues have been previously stained in bulk). The *mounting* with Canada balsam completes the process. It will be convenient to take all these processes in the foregoing order except that **section-cutting** and **staining** will have separate divisions, owing to their importance.

These various processes, as far as permanent mounting in Canada balsam is concerned, may be tabulated as shown on the opposite page.


Washing
For Uncut Objects.
For Sections.
Staining [and decolorizing]
Washing
Dehydrating
Clearing
Mounting in Canada balsam
[Staining in bulk to be done here]
Dehydrating, finishing with absolute alcohol
For Paraffin.
For Celloidin.
Clearing
Infiltrating
Sectioning
Flattening [and affixing sections to slide]
Xylol (to remove paraffin)
Absolute alcohol (to remove xylol)
Ether-alcohol
Thin celloidin
Thick celloidin
Embedding and hardening
Sectioning
[Affixing sections to slide]
Staining [and decolorizing]
Washing
Dehydrating, finishing with absolute alcohol
Clearing
Mounting in Canada balsam

Dissection.—Certain objects may need dissection by ordinary methods, and a few words on the subject may be interpolated here. Most dissections, and especially delicate dissections, are done under water, with, perhaps, a little methylated spirit added if the object has previously been soaking for some time in methylated spirit or alcohol. In some cases it will be necessary to fasten the object down, and this may be done with pins on a weighted piece of cork placed inside the dissecting-dish, or by running paraffin or some such compound into the bottom. Watch-glasses with flat bottoms make useful dissecting dishes. Two or three needles set in light wooden handles will be required, with both straight and bent points, and these can readily be manufactured at home, or purchased for a few pence. In buying dissecting knives, those with ivory handles should be chosen; they only cost 1s. 9d. each, as against 1s. 6d. for the ebony-handled ones, while the latter are so brittle as to break with very little pressure. There are a good many shapes of blades sold, but perhaps the most generally useful are the usual scalpel forms, the spear, and the spatulate-shaped ones. Forceps may be either steel, brass, or nickel, but perhaps plated steel ones are best, and they should, of course, be carefully kept clear of rust. A few camel-hair brushes are also necessary, and a pair of fine scissors. Insects generally require soaking in a 10 per cent. solution of sodium or potassium hydrate (caustic potash) for periods varying from an hour or two up to a week. Too much soaking will destroy the object, and also render it too transparent after mounting, whilst too little may leave it hard and difficult to deal with. A little thought and attention will therefore be necessary, and slight pressure with a blunt needle will tell whether the object is sufficiently

soaked. In the case of large insects, like cockroaches, they should be soaked for several days until they begin to give off an unpleasant smell. The alkali must then be removed by soaking in several changes of clean water. The inside of the insect can be got rid of by gentle treatment with the camel-hair brushes. Plant subjects are usually softened by long soaking in water.

Very small dissections are best done in cedar oil. Clove oil has a tendency to make objects brittle.

Normal saline solution (0·75 per cent. of common salt) is a most useful medium for many dissections.

Fixation.—*Killing*, *fixing*, and *hardening* are often included under the head of 'fixing,' though *killing* is the destroying of the life of the animal or tissue, whilst *hardening* hardens the tissues, rendering them able to stand subsequent treatment, and *fixation* preserves them in a normal condition, and incidentally tends more or less to differentiate them optically. The three processes are not always identical, alcohol, for instance, which is an excellent hardening agent, being an inefficient fixing one. The killing may be part of the fixing and hardening process, but in the case of small animal organisms which are required to be fixed in an extended position it may need to be preceded by slow narcotization with cocaine or chloroform, as in the methods for killing rotifers devised by Mr. C. F. Rousselet. Marine animals are rather troublesome to fix, and require careful washing away of the sea-water.

A suitable fixative must act quickly and well, in order to kill quickly, to maintain existing relations, and to prevent post-mortem changes, which often take place with more rapidity than would be imagined; it must not distort the tissues either by shrinkage or by swelling; it must penetrate well; and it must not interfere with

subsequent staining processes. It should not have a tendency to form artefacts, whether in the shape of precipitates or otherwise. It may be as well to say at once that no single reagent fulfils all these requirements, and that the best reagents are, therefore, mixtures, mostly with acid reactions. Much judgment is required in selecting suitable fixatives for various objects.

The aim of hardening is to enable good sections to be cut, and for this purpose alcohol is most generally used.

All fixatives must be washed out thoroughly in plenty of liquid; if aqueous solutions, such as osmic and chromic acids, they are washed out in water or weak alcohol; if alcoholic solutions, in alcohol of about the same percentage. Picric and acetic acids, and acetic alcohol, as will be seen later, are washed out in alcohol of at least 70 per cent., water counteracting their effect.

The tissues to be fixed must be living or fresh at the time of application of the reagent, and, as rapid penetration is necessary, as small pieces of tissue as possible should be used, and as large a quantity of fixative (say twenty times the bulk of the object). The solution will probably need changing at least once. The tissues are best suspended at the top of the jar in a muslin bag, or in one of Fairchild's perforated porcelain cylinders (which are small enough to float from thin corks), in order that any precipitate may gravitate away.

A great many reagents are used for fixation, but a few of those in common use can alone be dealt with here. On proper fixation depends in great measure the final result, and too much care cannot be taken in carrying it out.

Both fixation and subsequent washing are largely accelerated by heat. Heat alone is seldom satisfactory as a fixative, except in bacteriological work. Boiling

water is useful mostly when a hurried method is required, as in post-mortem work, and generally gives inferior results.

Osmic Acid.—This is used either as vapour or in weak aqueous solution, and also in combination with other reagents, such as chromic and acetic acid. It is useful for delicate tissues, acts rapidly, and kills quickly, but has poor penetrative powers when used alone. It is useful for cytoplasm, but is unsatisfactory for nuclear structures, unless combined with acetic acid. It stains fatty substances black or brown. It is very volatile, and in many people sets up an irritating conjunctivitis, or catarrhal condition, so should be used with care. It should carefully be kept free from dust, otherwise it is quickly reduced, and it is best to keep it in the dark. A 1 per cent. aqueous solution is generally used, diluted at the time, if need be, with a few volumes of distilled water. Tissues are placed in this solution for six hours or more, and the bottle kept well corked and in the dark. Sometimes only the vapour of the 1 per cent. solution is used, the slide being merely brought over the mouth of the bottle, or the tissues pinned to the cork, and kept there until they turn brown, which may take half a minute or several hours. The tissues are then washed well with *water*, and may be stained with hæmatoxylin or any of the aniline stains. Carmine does not stain well after osmic acid, unless the tissues are bleached first. Osmic acid is often combined with chromic acid, as in Flemming's solution, and the latter counteracts the blackening of fatty tissues.

Picric Acid.—A saturated aqueous solution (about 1 in 75 in the cold) is generally used, or sometimes a 1 per cent. solution, in 70 per cent. alcohol. This reagent penetrates well, but fixes and hardens indifferently.

On the other hand, it is easy to use, and does not tend to make tissues brittle. It gives very good results when combined with formalin. Its action is rapid, varying from a minute to twenty-four hours, according to the size of the object; but as it is quickly washed out by water, the subsequent washing must be done with 70 per cent. alcohol, and, generally speaking, only alcoholic stains should be used. Picric acid stains skin, bone, connective tissue, chitin, etc., brown. It dissolves calcareous substances.

Chromic Acid.—A 1 per cent. aqueous (not alcoholic) solution is most generally useful, made up as required from a 10 per cent. stock solution. Chromic acid hardens quickly and well, but penetrates badly, and tends to make the tissues brittle if left too long. The object must be small—say ½-inch cube—and not less than 100 c.c. of chromic acid should be used, and the fluid changed more than once. It is sometimes advisable to begin with weaker solutions, and the time required for hardening may be from a day to two weeks. The chromic acid must be very thoroughly washed out in running water for twenty-four hours, or it tends to form a fine precipitate when put into alcohol. Aqueous stains may be used, especially hæmatoxylin or safranin. Carmine in particular stains badly with all chromic acid reagents or its salts. Chromic acid and its salts are much used for botanical work.

Flemming's Solution is a combination of chromic acid, acetic acid, and osmic acid according to the following formula:

Chromic acid (10 per cent. solution) ..	7 c.c.
Osmic acid (1 per cent. solution) ..	40 c.c.
Glacial acetic acid	5 c.c.
Distilled water	48 c.c.

It is useful for delicate tissues, and for minute structural details, such as mitoses, but does not keep well, and is less used now than formerly, as it penetrates badly, and therefore tends to over-harden the outer parts of the tissues without properly reaching the centre. It should be used for small objects only. Objects are immersed for twenty-four hours, then washed well and carefully in water, placed in 75 per cent. alcohol, and, if necessary, kept in stronger alcohol. Owing to the osmic acid, it browns or blackens fat.

Potassium Bichromate.—Perhaps the most generally useful of all hardening agents (especially for nervous tissue), as it does not make tissues brittle. It is mostly used as a 2 to 5 per cent. aqueous solution, beginning with the weaker solution. Used alone, it penetrates slowly, and does not fix well, and is therefore best used with the addition of acetic acid, as follows:

Potassium bichromate	3 grams.
Glacial acetic acid	5 c.c.
Water	95 c.c.

This reagent is very useful for embryos. Small objects will require one or two days; larger ones may require some weeks, and it is advisable to change the fluid at least once. Washing must be done very thoroughly in water, and then the object passed through alcohols of increasing strength. Hæmatoxylin or the coal-tar stains can be used, but if carmine be selected, the treatment with alcohol must come after the staining.

Müller's Fluid is a good hardening agent for delicate structures, but is not so much used now as formerly. It penetrates evenly and well, so that it is suitable for large objects, but, like all the bichromate solutions, it discolours the tissues somewhat, and has the more serious

fault of producing retiform or granular deposits. The following is a common formula:

Potassium bichromate	2–3 grams.
Sodium sulphate	1 gram.
Water	100 c.c.

Tissues require at least twenty times as much Müller's fluid, and the latter must be changed at the end of twenty-four hours, again three days later, and again after another week. From two to six weeks are necessary. The tissues are then well washed for twenty-four to forty-eight hours in running water until it is of a pale lemon colour, and dehydrated in graduated alcohols. It is well to carry out the hardening in the dark.

Mercury Perchloride (*Corrosive Sublimate*).—A good reagent for general work other than cytology, though not very easy to work with. It fixes well and rapidly, and hardens and penetrates well, but it shrinks cells very badly, and makes tissues very brittle, nor does it differentiate well. Large quantities should be used in increasing strengths. A saturated solution (6 to 7 per cent.—Lee), with 1 per cent. of glacial acetic acid added, is commonly used. The object should be suspended in the reagent, as already described, and removed when it becomes opaque. The time required may be from a few minutes, or even less, to two hours, and the object is then washed well in water or 70 per cent. alcohol, preferably containing iodine-potassium iodide. If insufficiently washed or kept too long in alcohol, the tissues are very brittle, take stains less sharply, and tend to show annoying precipitates. Any stain can be used, especially carmine. It will be borne in mind that iron or steel needles or instruments must not be put into mercury perchloride.

Gilson's Fluid is very useful for beginners, as it is

easily used, and objects can be left in it for a considerable time without damage. The time required generally varies from fifteen minutes to six hours or more. It penetrates well, and objects take stains well afterwards. Professor Gilson's formula is:

Nitric acid	1·5 c.c.
Glacial acetic acid	0·5 c.c.
Corrosive sublimate	2 grams.
Alcohol (60 per cent.)	10 c.c.
Distilled water	88 c.c.

For marine animals a few crystals of iodine added will prevent precipitates (Lee); but if they are found, they may be washed away with water containing a little tincture of iodine.

Acetic Acid.—This is, as will have been observed, very valuable as an addition to other reagents, but is less useful when used alone, as under such circumstances it kills rather than hardens, and if used for long, tends to swell tissues badly. Its main use is therefore to counteract shrinkage, to neutralize alkalinity, and to assist differentiation. It is used in 0·2 to 1 per cent. solutions. With alcohol, in the proportion of 1 of *glacial* acetic acid to 3 of alcohol, it forms a very penetrating and rapid fixative, requiring from six to twelve hours, preserving both nuclei and protoplasm, and it can be followed by any stain. It must be washed out with 70 per cent., or preferably with absolute alcohol, however, and all aqueous stains avoided.

Alcohol used alone is a bad fixative, but an excellent hardening agent, especially after a good fixative, and this is its greatest use. It dissolves fat, and oxidizes readily in the presence of chromates and similar reagents. As a hardening agent 70 per cent. alcohol is useful, and it is well to begin with weak solutions; but

90 to 95 per cent. alcohol is the most useful of all. Large amounts are advisable, with frequent change, or suspension of the object near the top of the jar. The time occupied may vary from twenty-four hours to several weeks.

Formic Aldehyde (*Formaldehyde*).—Formalin or formol is used to denote a 40 per cent. solution in water. This reagent has become very popular, as it fixes and hardens well, and does not make the tissues brittle; and it is also a powerful antiseptic, but it is somewhat uncertain in its action, and is certainly inferior to alcohol—as a hardening medium, at least. For cytology it does not give good results. A 0·5 to 4 per cent. solution is generally used, and the object then transferred to alcohols of increasing strength. It can be followed by any stain. Formalin is a strong reducing agent, and must not be used with chromic or osmic acid. It does not keep well, as in time it decomposes with the formation of a white precipitate. Its vapour, like that of osmic acid, is very irritating to the eyes and nose, though in a less degree, and it quickly hardens the skin of the fingers.

Formalin is combined with Müller's fluid in the proportion of 1 part of formol to from 10 to 100 of Müller's fluid. The fluid must be made up as required. Fixation requires twelve hours and upwards, and the reagent must be well washed out in running water.

Dehydration.—The most generally useful method of extracting water from tissues, whether it be inherent or acquired—*dehydrating*, as it is technically called—is by means of alcohol. A robust section may be put into strong alcohol direct from water, but in most cases the section will become distorted or torn by such treatment, and, therefore, graduated alcohols are used. A suitable

graduation would be to have alcohols of 30 per cent., 50 per cent., 75 per cent., and 95 per cent. strengths. Elaborate tables have been made out for calculating these strengths, but they are in most cases not really necessary. One or two points should be borne in mind: firstly, that the amount of alcohol should be ample at each stage; secondly, that each process should not be hurried; thirdly, that alcohol rapidly absorbs water from the air. It is well, therefore, to provide stoppered stock bottles of the above strengths, and also short, wide-mouthed, stoppered bottles of such a size that a 3 by 1 inch slide can be inserted entire. These last bottles can be used a good many times, unless work requiring special care is being done; but in all cases the bottles should be kept stoppered, and if alcohol is used in a watch-glass, another watch-glass should be placed above it.

Guyer gives a convenient and nearly correct working rule to obtain a given percentage of alcohol—*i.e.*, to subtract the percentage of the alcohol required from the percentage of the alcohol to be diluted; the difference is the water to be added. In practice, fill the graduated cylinder to the same number as the percentage required—*e.g.*, 35—with the alcohol to be diluted—*e.g.*, 95—and then fill up to the percentage of the latter—*e.g.*, to 95—with distilled water.

Absolute alcohol is, fortunately, very rarely necessary, as it is very expensive, the clearing agents being able to remove a small amount of water, so that 95 to 98 per cent. alcohol is quite sufficient, provided the warning already given be acted upon, and the bottle kept rigidly stoppered. It is surprising how soon so strong an alcohol will absorb water, and thus spoil a series of sections. For most practical purposes good undiluted methylated

spirit may take the place of 95 per cent. alcohol, but it must form no precipitate with water.

The time required for each stage of dehydration varies with the size of the section. Thin sections that have been cut in a microtome may not require more than one to three minutes, whilst other tissues which contain much water require as much as twenty-four hours. The worker will learn to exercise his own judgment, remembering that an extension of time will generally do no harm, except, perhaps, to make the section a little more brittle. Of course, alcohol discharges the colour from many stains, but such matters will be left over until staining processes themselves are discussed. The last strong alcohol should always have full time given to it.

The strains undergone by the various tissues even by the use of graduated alcohols are illustrated by the diffusion currents plainly set up when the section is transferred from one strength to the other. The currents are visible to the eye, and the section often spins round with vigour. For this reason very delicate tissues may even need the use of a special diffusion apparatus, such as is described in Lee's 'Microtomist's Vade-Mecum,' which consists of a tube closed with a cork at one end and with a diaphragm of chamois leather at the other end. The section is put into this tube in the alcohol of the weaker strength, and then immersed in the stronger alcohol, the diaphragm allowing a gradual and safe diffusion between the two.

Clearing.—The water having been got rid of by means of alcohol, this last must itself be got rid of, the process being known as *clearing*. Strictly speaking, as observed by Lee, a clearing agent is a liquid which has a refractive index very nearly as high as the object to be cleared, and which causes the object to become proportionately trans-

parent, but it so happens that all reagents used for extracting alcohol have also high refractive indices, and, therefore, the term is used indiscriminately to imply a fluid which both clears and extracts alcohol.

The clearing agents in commonest use are given below, with their respective refractive indices, taken from Lee:

Oil of bergamot	1·464
Oil of turpentine	1·473
Xylol	1·497
Cedar-wood oil (unthickened)	1·510
Oil of cloves	1·533
Aniline oil	1·580

Of these, it may be stated that oil of cloves and aniline oil will remove 90 per cent. alcohol, or even below this; whilst oil of bergamot comes next in efficiency. Xylol will absorb an alcohol of not less than 95 per cent., but is slow in action. Cedar-wood oil is superior to xylol in this respect. Chloroform takes up alcohol of less than 95 per cent., but is too volatile. Xylol, benzole, chloroform, clove oil, and turpentine dissolve paraffin well, and the former is, therefore, much used for preparing sections that have been cut in paraffin for mounting in Canada balsam. Aniline oil is most used for preparing celloidin sections, because of its solvent action on that substance. Cedar-wood oil and oil of bergamot clear celloidin well without dissolving it. These last have also only slight action upon aniline stains, which are rapidly discharged by clove oil. Clove oil also dissolves celloidin. Turpentine shrinks the tissues very badly, and makes them very brittle; oil of cloves has the same tendency in a less degree, but is an excellent medium in which to make fine dissections; xylol clears well, but slowly; cedar oil is very penetrating, most useful in preparing sections for paraffin embedding, and seldom

causes shrinkage in the most delicate tissues, whilst it leaves them very pliable, but it does not extract water well much below 95 per cent. alcohol. On the whole, cedar oil is the most reliable clearing agent, and the one which will cause least loss of sections. Moreover, tissues can be left in it for an almost unlimited time without harm. One clearing agent which has not yet been mentioned is carbolic acid. It clears sections in which there is still a good deal of water excellently, but they are apt to shrink in the Canada balsam after mounting. Mixed with xylol in the proportion of 3 of xylol to 1 of carbolic acid (in crystals), it forms a useful clearing agent, but requires care in handling. After cedar oil, xylol is the most useful, and it is pleasant to work with, but, of course, evaporates quickly.

So much for the choice of clearing agent. The section, having been carefully dehydrated, is then put into the selected de-alcoholizing and clearing agent for a variable time until clear and transparent, this being the last stage of all before the actual mounting in Canada balsam. There are one or two points still to be mentioned, however. The clearing agents must be kept scrupulously free from moisture, even that of the breath or of the atmosphere, or they will become cloudy and inefficient. The more nearly the refractive index approaches that of the tissues, the more transparent will the object become, and *vice versâ*, but the less contrast will, of course, be shown, especially when mounted in Canada balsam. In transferring from the last alcohol to the clearing agent no more of the former should be removed than is unavoidable, and it is a good plan to touch the edge of the section momentarily in passing against some smooth filter-paper (blotting-paper is too fluffy, and may give trouble with loose fibres) to get rid

of the excess. The sections must on no account be allowed to get dry, or they will be irretrievably ruined. To prevent shrinkage of delicate tissues, Mr. Lee's plan may be adopted—*i.e.*, the placing of the clearing medium in a tube or watch-glass *under* the alcohol by means of a pipette, so as to form two distinct layers. The section is then gently inserted, and will float at the junction of the two liquids until it is saturated with the clearing agent, when it will slowly sink to the bottom, after which the alcohol can be pipetted off.

There are many ways of transferring sections during the foregoing processes. Small German silver and other spatulæ are sold for the purpose, but are apt to get greasy, and so give trouble. A clean cover-glass held in forceps is convenient in many ways, but for sections a thin glass rod is, perhaps, best of all. The section clings to the rod when it is lifted, and with ordinary care there is no danger of tearing the tissues.

Mounting Media.—It will be convenient to deal next with mounting media. These are mainly of two kinds—aqueous media and resinous media. The former are comparatively simple and straightforward, but are seldom suitable for permanent mounts; the latter are excellent, but require care, some trouble, and a little knowledge. Amongst the aqueous media we may place the most useful of all—namely, those into which glycerine enters, which is miscible in all proportions with water, and in which, therefore, sections may be mounted which contain water; whilst in resinous media, of which Canada balsam is the most universally used, water must be rigidly eschewed. It is the neglect of this latter principle, or its careless observance in the various prior processes, which is responsible for so many failures in mounting in Canada balsam.

Canada Balsam.—Canada balsam has a considerably higher refractive index than glycerine (1·535 for Canada balsam as against 1·456 for pure glycerine, and 1·397 for glycerine and water in equal proportions), and as mounting media should be selected to give as great contrast as possible between the tissues and the mounting medium, tissues which have a low refractive index requiring a medium of high refractive index, and tissues of high refractive index requiring a medium of low refractive index, this fact is worth bearing in mind. But Canada balsam, being a resinous medium, is of all media the most preservative for the object, and the most permanent. It is necessary to dissolve the balsam in some suitable solvent, and those generally used are turpentine, chloroform, xylol, or benzole, more especially the last two. Turpentine balsam sets more slowly than any of the others, and the mount has, therefore, less tendency to become brittle, and Mr. Lee points out that the index of visibility is retained longer with this solvent than with those which are more volatile; but it is, unfortunately, injurious to alum-hæmatein stains, whilst the chloroform solutions have a tendency to turn brown with age. It may be added that all solvents of Canada balsam are injurious to the coal-tar stains, and prevent their permanency, so that the selection of both stain and mounting medium requires some consideration. Dr. Mann likewise points out that the balsam itself must be neutral, the commercial balsams being always acid, and, accordingly, discharging the basic dyes. For this reason it is best to buy Canada balsam from the opticians, especially from those who stock the well-known preparations of Grübler. The author personally prefers to use balsam dissolved in benzole, but the benzole must be pure, and absolutely free from water.

It has a rather hign refractive index, and it sets somewhat quicker than xylol, but this is generally an advántage rather than otherwise, and benzole is more stable than xylol with regard to alkalies.

Canada balsam, whatever the solvent, should be of the thickness of syrup. It should be kept in a capped bottle, the bottle having a fairly wide mouth, and being provided with a glass rod. The cap should be kept on the bottle as much as possible, in order to minimize the evaporation of the solvent; but more solvent can be added if necessary. The older the balsam the better. It absorbs air-bubbles rapidly, and the thinner the solution the more rapidly will these oxidative processes go on, so that minute air-bubbles in a mount can generally be neglected. Stress has been laid on the fact that all moisture must be rigidly guarded against, not only in the object which is to be mounted, but even by such means as breathing on the cover-glass, a plan which has, unfortunately, even been recommended.

The actual mounting in Canada balsam is simple enough. We will assume that the section has received a final soaking in cedar oil. It is then generally transferred to a slide, a drop of balsam put on it, and a cover-glass gently lowered, one edge first, upon the balsam, so that the balsam spreads evenly out beneath. It requires some little practice to judge the size of the drop, but any excess can be removed when the slide is dry—say some days, or perhaps even weeks, later—by chipping it with a penknife, and then by rubbing the edges gently with the corner of a soft rag moistened with benzole or some other solvent. If the addition of the cover-glass is neatly done, any air-bubbles will be carried away to the side.

In many cases the weight of the cover-glass is itself

sufficient, but it is often of service to press lightly upon the cover-glass with the point of a needle, so as to squeeze out any excess of balsam, and to insure a flat mount. Springy sections may require pressure for a prolonged time, and small wire springs are sold for the purpose, as well as more elaborate adjustable apparatus. Any small weight may be used, such as lead type.

Mounting may in many cases be done upon the cover-glass rather than the slide, and when the section is affixed to the latter by one of the processes to be hereafter described, great economy of reagents results from this method, together with more convenience in handling.

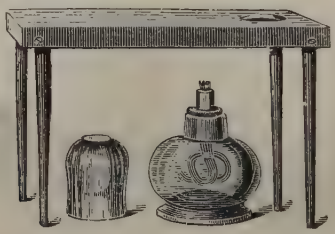


FIG. 113.—MOUNTING-TABLE.

The use of a mounting-table (Fig. 113) is practically never seen in medical and scientific laboratories, but it is of much service, especially in making cells, and in freeing tissues from air-bubbles.

Glycerine is seldom used pure, but generally with a certain amount of water. Its great drawback is its property of absorbing water and its solvent action upon the various cements which are used to ring round the preparation and to enclose it in an air-tight cell. Its other great drawback lies in the fact that it will not dry or harden. The result is that glycerine mounts can scarcely be said to be permanent, and are a constant

source of trouble to the owner of a cabinet, and slides made up with this medium require constant watching, and sometimes re-ringing. It is, therefore, seldom used for other than temporary mounts. A further point to be noticed is that glycerine acts as a solvent on calcareous substances.

In favour of glycerine may be mentioned its convenience, its ready applicability to many objects, with a minimum of preparation, and its refractive index, which makes it of good differentiating power. It may be noted that it largely increases the transparency of organic substances, and, though this is often advantageous, it may so far diminish contrast as to make them indifferent mounts.

Glycerine, as an aqueous medium, requires objects to be transferred into it direct from water, being thus in marked contrast to Canada balsam. The glycerine may be used pure, or diluted with water, the former having the higher refractive index, and thus giving the greatest differentiation. *Glycerine, alcohol, and water* also make a useful mounting medium for delicate objects, in the following proportions:

Glycerine	1 oz.
Alcohol	1 oz.
Water	2 ozs.

Farrant's Solution is a mixture of glycerine, water, and gum, and is more convenient than ordinary glycerine, as it sets at the edges. It may be made up as follows:

Gum arabic	2 ozs.
Glycerine	2 ozs.
Water	1 oz.

(To be kept in a stoppered bottle, with a lump of camphor or some crystals of carbolic acid.)

Glycerine Jelly is semi-solid when cool, and requires to be melted before use, either by putting the bottle in a cup of hot water, or by heating the slide on a hot mounting-table, and putting a suitable piece of the jelly on it, as required. Any air-bubbles should be skimmed off before the cover-glass is put on, and as the glycerine jelly will only solidify by cooling, and can be re-heated, there is no need to hurry the process. The glycerine jelly sets very rapidly as the slide cools, but the whole slide may with advantage be put aside for a few hours to set. The excess of jelly around the cover-glass may then be removed with a penknife, and the whole slide cleaned by dipping in water, finally polishing with a bit of rag. Glycerine jelly is often used when mounting in built-up cells, but before doing this it is advisable to run a wetted camel-hair brush around the cell to make sure that no air-bubbles may cling to the sides or bottom. The following is a good formula, though it will be safest for most workers to buy it ready made at the optician's:

Water	3 ozs.
Glycerine	$3\frac{1}{2}$ ozs.
Gelatine	$\frac{1}{2}$ oz.
Carbolic acid	1 drachm.

(Dissolve the gelatine in the water, and add the glycerine and carbolic acid. Warm for a quarter of an hour, stirring well, and taking care that the temperature is not raised higher than 75° C. or the gelatine will become changed, and refuse to harden in use. Filter through fine, hot flannel.)

In mounting in all glycerine media the object must be carefully soaked in water. One of the most troublesome matters concerned with glycerine mounts is their

non-absorption of air-bubbles, which are often very vexatious. It is, therefore, wise in many cases to soak the objects in water which has been recently boiled, and from which, therefore, the air in solution has been largely expelled, so that it may re-absorb air from the object. In certain cases an air-pump may be used. The object may then be transferred to a mixture of glycerine and water—say one-third of the former—for a further soak, or may be at once mounted in the glycerine medium, either with or without the use of the hot plate. Bubbles are often introduced when the cover-glass is added. Breathing on the latter will generally obviate this.

It is advisable to ring all glycerine mounts with a ring of glycerine jelly, and to cover this with a ring or two of gold-size, Ward's or Bell's cement, or marine glue, though the author confesses to having no love for the latter. Any excess of glycerine can be removed with water and a brush, and it is necessary to make sure that the edges which the cement overlaps are quite free from glycerine or water, or the former will not adhere properly.

Pressure on the cover-glass should be avoided as far as possible when making mounts in any of the glycerine media, and a further safeguard is the leaving of a small air-bubble under the cover-glass with which to counteract changes in temperature. This method, though it looks untidy, works well in practice, and in the slide cabinet of the Royal Microscopical Society (of which the author was at one time Curator) are slides which have been treated in this way, and which have been long mounted. The preliminary soaking must not be neglected, and in many cases the object should soak in glycerine of the same strength as that in which it is to be ultimately mounted, or the subsequent swelling may assist in loosening the cement.

Alcohol attacks cement, and, therefore, glycerine, alcohol, and water—a formula for which has already been given—is used mainly for delicate objects whose sudden immersion in strong glycerine would be harmful. By refraining from closing the mount for a few days the alcohol evaporates, and the glycerine and water are left.

Slides and Cover-Glasses.—Wooden and paper-covered slips are now rarely used, the 3 by 1 inch glass slide being universal, except for very large sections. Slides can be obtained in which a circular or oval hollow has been ground, and these are very useful for thick sections, or those in which pressure must be avoided. Deeper cells must be built up, as will be explained presently.

All slides and cover-glasses, even though new and unused, must be scrupulously clean, especially when serial sections are to be mounted on them. Mr. Lee recommends an ounce each of sulphuric acid and of potassium bichromate, in 8 or 10 ounces of water; but a fairly strong and hot solution of Hudson's soap is usually quite satisfactory, if followed by careful rinsing with clean water and rubbing with a glass cloth or an old cambric handkerchief, and subsequent immersion in methylated spirit. Cover-glasses are treated in the same way, and should be kept in methylated spirit with a trace of HCl in it until required. Any suspicion of grease must be avoided, and neither slides nor cover-glasses should be touched with the fingers, nor should they be breathed upon, except as previously suggested. A good test of the cleanliness of a slide is its ability to take a film of water uniformly when it is applied with a brush.

Cover-glasses are now always bought, and are made in England by one firm only. They may be round, or square, or oblong. The round cover-glasses are but slightly more expensive than the square ones, and make

much neater mounts, besides being easier to ring with enclosing cements and varnishes. They are sold of various thicknesses, of which those of about 0·005 inch and 0·01 inch are the most generally useful, the former for immersion lenses and the latter for lower powers. Objectives are corrected for a definite thickness of cover-glass and of tube-length, the former varying, according to the maker, from 0·006 inch to 0·008 inch, so that it would be advisable to use the thinner cover-glasses always, were it not for their extreme fragility. It is worth noting that in the modern short-tubed microscope there is little margin for correction of errors due to *thicker* cover-glasses, which necessitate shortening the tube. The most convenient sizes for the circular cover-glasses are $\frac{7}{8}$ inch, $\frac{3}{4}$ inch, and $\frac{5}{8}$ inch, and for the oblong glasses $1\frac{1}{2}$ inch by $\frac{3}{4}$ inch, and 2 inch by $\frac{7}{8}$ inch. The cleaning of all cover-glasses is much facilitated by having a block of wood covered with chamois leather, upon which they can lie flat without fear of breakage.

Mounting Opaque Objects and Cell-Making.—Opaque objects, for illumination by reflected light, are generally mounted dry. The procedure is comparatively simple, but in most cases requires the building up of a cell to contain the object. The shallowest cells are merely a ring of varnish or gold-size, of the requisite depth, put on by means of a turn-table (Fig. 114). The rotating plate has concentric rings engraved on its surface, to assist in centring the slide. 'Self-centring' turn-tables are not recommended. The author prefers gold-size for nearly all cell-making, but others use black enamel, especially 'Club Enamel,' black Japan, Brunswick black, white zinc cement, Bell's cement, Ward's brown cement, marine glue, or Hollis's liquid glue. Brunswick black and white zinc cement are excellent for final

coatings for all mounts, but are lacking in adhesive property for cell-making, and marine glue (which must be applied hot) is an abomination. Gold-size is the most generally useful and the pleasantest to work with, but it must be rather old and not too thin, and for the strongest cells Ward's brown cement is, perhaps, the most trustworthy. Hollis's liquid glue is useful for ringing slides which will be examined with oil-immersion lenses.

In building up a cell it is important to have the size or cement flowing freely from the brush. The brush

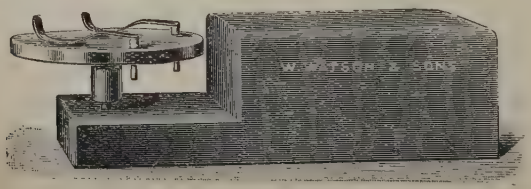


FIG. 114.—TURN-TABLE.

should be of sable, and of moderate size; the tip only is used, and too much should not be taken up at a time. The table should not rotate too quickly. In particular, each ring must be allowed to dry thoroughly before another is applied. The rings should be of such a size that the proposed cover-glass does not quite reach to their outer edge.

Deeper cells are most conveniently made by utilizing the hollow-ground slides, which can be bought cheaply, with circular or oval concavities of various sizes. These cells are also most convenient for mounting thick objects in Canada balsam, glycerine, or fluid media.

Still deeper cells are built up with rings of tin, celluloid, gutta-percha, or glass. Gutta-percha is rather

brittle, and does not fasten very well; glass is the best, but is not easy to get in quite such thin rings. Paper or cardboard rings cut out with a punch and soaked in paraffin are good, but without the paraffin would be too porous for liquid media. The cells can be fastened with any of the cements mentioned, subject to the remarks already made. The cell should be pressed gently down, and given a slight twisting motion to make it adhere more closely, and care should be taken that the ring of cement is complete all the way round.

For opaque mounts it is customary to cover the bottom of the cell with some opaque medium, such as Brunswick black, or black paper, or both. The black background should be dull. Suitable discs can be easily cut from dull black paper, such as is used for masks for lantern-slides. The drawback to such a background is that, whilst it enhances the effect when the object is illuminated from above, it renders the use of a Lieberkühn or similar means of illumination from below impossible, and the author's own practice is to mount all dry objects without a black background, and to have a slide or two carrying a disc of dull black paper to put below the opaque object when required. Of course, there is a slight amount of reflection from the slide.

Objects can be fastened in position with a little gum, which should not project beyond them. Very minute objects like pollen, etc., require a film of dilute gum on the background, which is allowed to dry, and then just breathed on to moisten it when mounting. The gum can be kept from becoming too brittle by adding a few drops of glycerine per ounce, and it is well also to add one or two drops of carbolic or some essential oil, to inhibit fungoid growths.

It is important to make sure that all cement or gum

used is absolutely dry before the cover-glass is finally applied, or a fine dew will settle upon the cover-glass and spoil the mount.

A final ring of good size is then run on, and this last should be allowed to dry until it is just sticky only, when the cover-glass may be lowered into place by means of a pair of forceps, and the edges pressed gently down, care being taken that the cover-glass adheres all round its edges. Finally, the slide may be finished by a coat of Brunswick black over all, just covering the edge of the cover-glass.

Ringing Slides.—Canada balsam slides do not necessarily need ringing, though the writer's own practice is to ring all his slides, but glycerine slides should be finished off with a couple of rings of gold-size. A ring of glycerine jelly may with advantage be put on before the gold-size is applied. The process is very similar to that of cell-making. The slide is centred upon the turn-table, centring by means of the cover-glass and not by the slide, and a ring of gold-size run round the edge of the cover-glass. Care must be taken just to cover the edge of the latter, and not to overlap the slide too widely. Beginners generally take up too much gold-size in the brush. A neat ring is made by attention to this point, by turning the table at a moderate speed, and by raising the brush slightly towards the finish. Old gold-size is best, provided it will run easily. The second ring should be added after the first is thoroughly dry. Finally, a ring or two of Brunswick black or white zinc cement may be run over all as a neat finish.

For use with immersion lenses *all* mounts should for safety be carefully ringed with shellac cement or Hollis's liquid glue, or the cedar oil will dissolve the cement.

Cleaning Slides.—Spoilt slides, failures, etc., are best immersed in a strong hot solution of Hudson's soap, after which the slides and covers are well washed with warm water, rinsed with methylated spirit, and polished with an old handkerchief.

All slides should be carefully labelled as soon as completed, or much confusion will arise. The label should bear particulars (letters are quite sufficient) of the staining method and mounting medium employed, and of the date. The slides should be kept in proper boxes. The boxes should contain trays, so that the slides may lie flat, and these can be obtained very cheaply.

(*b*) Section-Cutting.

It has been thought most convenient to begin with mounting methods, and tacitly to assume that neither sectioning nor staining were required. However, the majority of objects require both, and these processes must now be dealt with in their due order. It is scarcely necessary to do more than allude in passing to merely mechanical means of separation, such as teasing with fine needles, or to dissecting, or to chemical dissociation by maceration.

As stated at the outset, most objects are too thick and too opaque to give good results when examined microscopically, and, therefore, thin sections or slices are required. Some sections can, of course, be cut by hand, and a little practice and patience will soon give great facility in this respect. A good razor is the only essential, but it is important that it should be sharp and in perfect condition. Hollow-ground razors are of little use. The best razor is one of the old-fashioned heavy English type, and a very suitable one can be bought almost anywhere. The beginner finds most

difficulty in keeping a good edge on his razor, and it is strange how often one sees the stropping done in just such a way as might best be calculated to destroy what edge there is on the razor, so a few words on the art of stropping may not come amiss. The author would advise that the ordinary leather strap should be rigidly avoided. If it must be used, it ought to be held quite taut. The best strops are those mounted upon handles, upon which the leather is carefully stretched to form a slightly convex surface from side to side. Such strops have, of course, one side of plain leather, and the other side covered with some kind of preparation. The latter should be used sparingly. The important points are to have the strop firmly supported at one end, either by a ring on a hook, or upon the table; to hold the strop firmly; to lay the razor flat upon the leather without any inclination whatsoever; to use moderate pressure; to strop the razor backwards; and above all, to turn it at the end of each stroke on its *back*, and not upon its edge. The turning of a razor upon its edge when stropping will ruin the best edge ever put upon it, and yet one frequently sees it done by inexperienced users. Moreover, it looks very easy when one sees a hair-dresser stropping a razor with a few lightning turns of his wrist, but those of us who do not aspire to professional skill of this sort, born of constant practice, will be well advised to strop slowly, carefully, and steadily, remembering that in too many cases we are far more likely to damage than to improve the edge of our razors.

Section-Cutting by Hand.—For actual section-cutting by hand the object is held between the forefinger and thumb in such a way that the palm of the hand lies towards the body, the fingers are bent round the object, and the tip of the thumb comes nearly horizontally

against and slightly lower than the middle joint of the first finger, this middle joint itself lying quite horizontally. Thus the object requiring cutting is held between the tip of the thumb and the middle joint of the forefinger; the tip of the thumb is just below the level of the cut of the razor, and so out of harm's way, whilst the middle joint of the forefinger forms a horizontal table to steady the razor in cutting. A dish of methylated spirit and water is kept close by, and into this the razor is dipped constantly, so as to flush both it and the object, whilst the razor is drawn steadily and slowly with a slicing motion towards the operator. The sections as they are cut are floated off into a small dish of mixed spirit and water. With a little practice wonderfully thin and regular sections can be cut in this way from suitable objects. As a means of practice a piece of dry elder-pith or a bit of the stem of a carrot can be recommended.

Hand-Microtomes.—Better and more regular sections can, of course, be cut by means of the hand-microtomes, of which there are many patterns offered for sale. They generally consist of a flat, horizontal plate of brass or glass, perforated with a hole in the centre, with a tube attached at right angles to contain the object and to serve as a handle (Fig. 115). The object is pushed through the hole, and is fed as required by means of a large-headed screw beneath it. Many of these simple microtomes have graduated screws for this last adjustment, so that the regularity of the thickness of the sections can be assured, and such thickness even calculated. Some are made to clamp on a table.

The object is generally fastened firmly in the feeding-tube by wedges of elder-pith, carrot, or cork, or even embedded in paraffin; other hand-microtomes have an

adjustable clamp at the side by which the object is held in place in a tight-fitting sleeve, which allows of the necessary movement up and down. The well-known Cathcart microtome is really a modification of these simple hand-microtomes, but it is fitted with such a clamp as just mentioned; it fastens on to the table, and instead of having a circular plate to serve as support to the razor it has two parallel side supports covered with glass. There is an even more elaborate pattern made (Fig. 116) in which the side supports are covered with brass, and

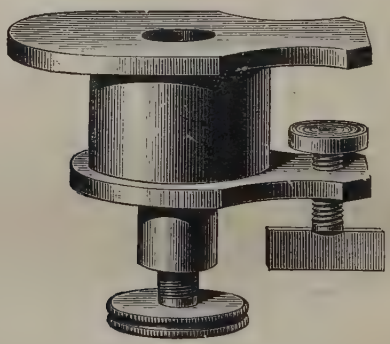


FIG. 115.—HAND-MICROTOME.

upon them runs a simple carrier to hold the razor rigid whilst it travels to and fro. Moreover, the screw-head for feeding has graduated teeth and an arrangement for recording the amount of movement, and an additional fitment consists of an interchangeable plate, bottle, and bellows for ether-freezing. The cheapest hand-microtomes can be purchased for a few shillings; the Cathcart microtome costs from £2 10s. to £3, according to its fittings. Even those who possess the more costly and elaborate microtomes, which will be described presently,

can find many uses for one of the simpler forms just mentioned.

The ordinary razor is generally used for all forms of hand section-cutting, but as many of the sections cut by hand are those which are too hard or too uneven in their consistency to be satisfactory when cut in more elaborate microtomes, it is well to provide oneself with a special strong razor-blade mounted in a rigid handle, such as

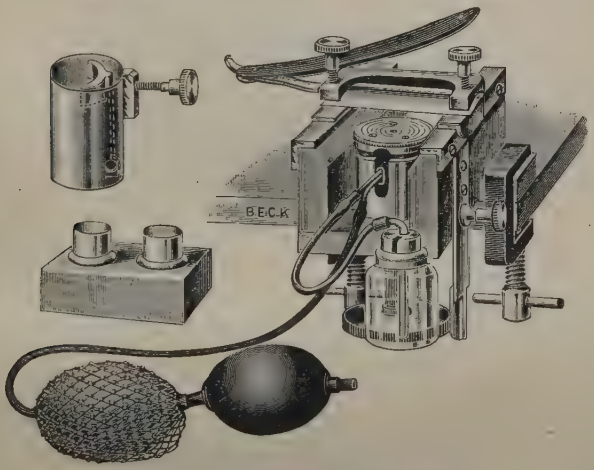


FIG. 116.—CATHCART MICROTOME.

most microscope dealers supply for about the price of an ordinary razor. For cutting very hard sections such as wood, nothing is better than a good plane-iron enclosed in a wooden handle, and one of the drawbacks to the more elaborate Cathcart microtome, with its brass slides, as against the old glass faces, is that this plane-iron cannot, of course, be used.

Precision Microtomes.—For the finest and most delicate work microtomes of great precision are necessary,

and these are now made in considerable variety by many makers. It is curious that, with the exception of the 'Cambridge Rocking Microtome' and of the Watson-Denne microtome, most of these are of Continental or American manufacture or design. The oldest of the types now in use is the Jung-Thoma microtome, based upon the Rivet model. It consists of a solid heavy casting, on the top of which are parallel slides in which a block runs, to which is clamped a projecting knife, and this block and knife are drawn by hand backwards and forwards on the slides. At the side of the casting, and thus beneath the knife, is another set of parallel slides which slope diagonally upwards. On these latter slides runs a carriage, which forms a carrier for the object, and the carrier is pushed onwards and, therefore, upwards on the slides by means of a screw, which is clamped in the requisite position, and turned by hand as required. By this means a fairly coarse thread can be given to the screw, and yet the resultant vertical movement of the object remains slight.

This instrument does not possess a mechanical feed, and the carrier has a tendency to jump slightly when hard or large sections have to be cut; but this form of microtome has done excellent work in its day, and is still to be seen in many of our laboratories.

The more recent types of microtomes, whilst retaining the principle of knife-block and slides as described above, are modified in that the object moves in bearings at right angles to the travel of the knife, the necessary delicacy of motion being obtained by having a very fine micrometer screw to feed the object-carrier. A simple form, suitable for students and clinicians, is sold by Bausch and Lomb, and is illustrated in Fig. 117. Freezing apparatus can be added. This microtome will cut

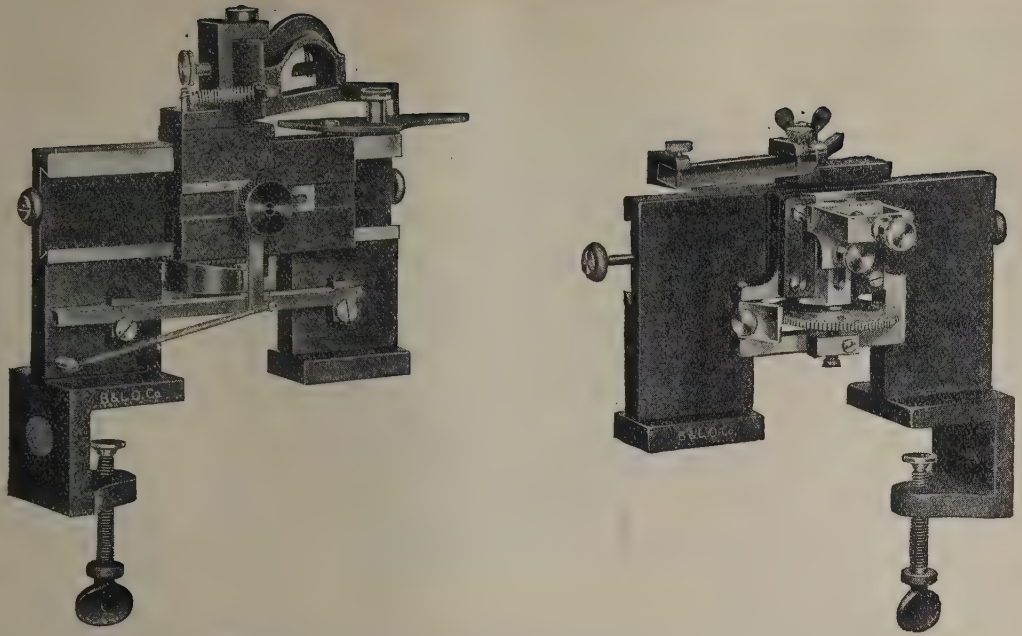


FIG. 117.—TWO VIEWS OF BAUSCH AND LOMB'S SIMPLIFIED CLINICAL MICROTOME.

sections 30×22 mm. and about 5μ in thickness. Larger models are obtainable. This pattern lends itself to many devices for automatic feed, either by hand or by an arrangement by which each return of the knife is made to actuate a ratchet and pawl movement, and so to raise the object a definite distance each time, the distance being capable of pre-adjustment within quite wide limits. In most cases, also, a mechanical move-

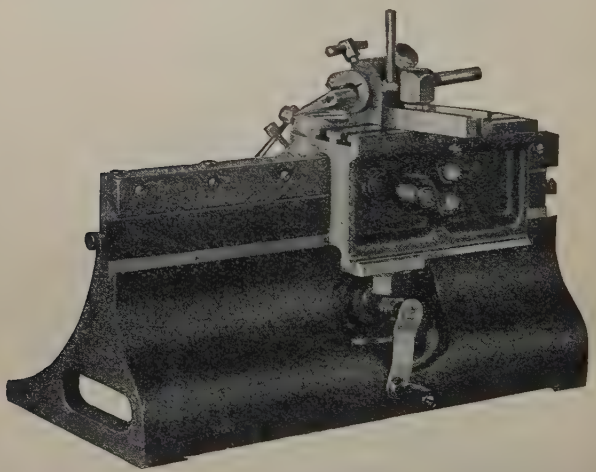


FIG. 118.—SPENCER MICROTOME WITH MECHANICAL FEED.

ment can be given to the knife. This type of microtome is exceedingly efficient, and cuts sections under varying conditions with great regularity. So fine a micrometer screw has, however, a tendency to wear, and the bearings of the carrier may also wear. It might be thought that the overhanging knife, supported as it is on one side only, would be liable to spring somewhat, but the weight of both carrying-block and knife almost entirely obviates this in practice. To most of these instruments modifi-

cations are fitted, by means of which sections can be cut when embedded in celloidin, or to enable them to be frozen in ether, ethyl chloride, or carbon dioxide gas. Microtomes, with many individual and ingenious modifications of one sort or another, are supplied by Baker, Beck, Watson, Schanze, Leitz, Reichert, Bausch and Lomb, The Spencer Lens Co. (Fig. 118), and other makers.

R. and J. Beck make a microtome on somewhat similar principles, designed by Professor Delépine, in which the knife runs on two slides set one on each side of the object; but it does not seem to have come into such general use, and it is, apparently, primarily designed for frozen sections. Bausch and Lomb make a somewhat similar type, in which the object moves on two such slides.

The Watson-Denne microscope is on the rotary principle, in which a hand-wheel works a connecting-rod and moves the object-holder, the knife being fixed.

Frequently only a few sections from an object are required, but in many cases it is necessary to take a series of sections right through so that the whole can be examined systematically. For instance, if one is studying the development of an early embryo, it is necessary to have complete sections through the embryo from one side to the other, losing none, and having all in orderly sequence, and such sections may easily run to 800 or 1,000 distinct sections. These are known as 'serial sections,' and though such a series can with skill and care be cut in the microtomes above described, it is much easier, as well as safer, to cut them in microtomes specially designed for the purpose. The 'Cambridge Rocking Microtome' (Fig. 119), made by the Cambridge Scientific Instrument Company, cuts such sections with great facility, and it is, one is glad to say, of English

manufacture and very moderate price. It is found in nearly all our English laboratories. The fault of the 'Cambridge Rocking Microtome' is that the rocking motion gives sections which, though uniform, are not quite flat, though this is a theoretical rather than a practical objection in nearly all cases; but to correct this a more costly form has been designed, which obviates this defect. Most workers, however, much prefer the simpler form, and find no disadvantage in its use. This

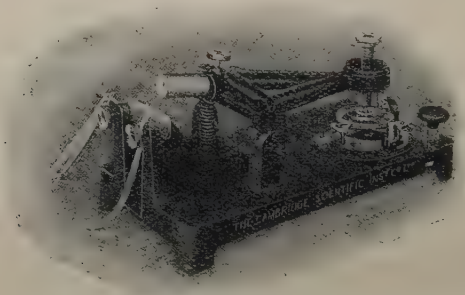


FIG. 119.—CAMBRIDGE ROCKING MICROTOME.

type of microtome is not able to cut sections of more than moderate size.

Another well-known serial-cutting microtome is the Minot, which is much used abroad and in America. It is worth noting that in all these types of serial instruments, unlike most of those formerly described, it is the object which moves and the knife which is fixed.

The 'Cambridge Rocking Microtome' requires only a simple razor; the other types require heavy knives of various shapes, all somewhat costly, and by no means easy to sharpen. A few hints will be given on this point before the suggestions on section-cutting are finished.

but it may be said here that the knives are generally supplied with thin hollow-ground edges for celloidin embedded objects, with less thin and less hollow-ground edges for paraffin, and with strong, straight edges for frozen sections (Fig. 121, p. 285).

Comparison of Celloidin, Paraffin, and Freezing Methods.—This brings us to the next point—as to the respective merits of celloidin, paraffin, and freezing methods for sections.

The *advantages* of the *freezing method* are that the tissues do not require long or careful preparation beforehand, and, therefore, that it can be utilized at short notice. The object is frozen to give it the necessary consistency and firmness to enable it to be cut by the knife, whilst retaining its parts in due position. Ether, ethyl chloride, or carbon dioxide gas can be used as the medium which, by its evaporation, will produce the necessary amount of cold. Ether is the most frequently used, and for this a simple and inexpensive microtome like the Cathcart does very well, but the fine orifice of the ether tube is rather apt to become blocked. Ethyl chloride used as a spray is convenient but expensive, and it is not easy to keep the tissues at the right degree of freezing. The best medium of all is carbon dioxide in cylinders, and microtomes are made now to screw on to such cylinders. The initial cost is somewhat great, and the cylinders are, of course, far from portable, but the cost of compressed carbon dioxide is less than that of pure ether.

The *disadvantages* of the freezing process are that delicate tissues are apt to tear, that thin and regular sections cannot be obtained, and also that the freezing process itself may alter the arrangement of the cellular structures. On the other hand, for the rapid production of

pathological sections without prior preparation either in the post-mortem room, or even during the actual carrying out of an operation (a method which is being increasingly utilized by some of our best-known surgeons), the freezing method is most useful, and the sections so produced have the further advantage of staining readily. There are some tissues, also, such as those containing minute fat globules, which would be spoiled by any other method of treatment.

The *advantages* of the *celloidin method* are that the object does not require any heating, and that there is accordingly no risk of over-heating. This is sometimes an important matter with delicate objects, nor is it necessary to clear the objects beforehand. There is thus no danger of over-heating on the one hand, or of distortion or shrinking on the other. The transparent celloidin makes the orientating of objects, when required, an easy matter; indeed, with very transparent objects it may be necessary to stain them slightly to enable them to be seen and recognized. The process requires little attention, and does not need watching, unlike paraffin embedding. It is also easy of application and of manipulation during the various stages of embedding, whilst the tissues are not likely to be damaged by being left too long in the solutions. For very brittle sections which require firm support celloidin has a distinct advantage, and larger pieces can be cut than by any of the other methods. Finally, celloidin does not require removal from the tissues before mounting, so that it holds them well together. The *disadvantages* of the method are that the process is very slow, generally taking about three days and often much longer; that the sections are not easy to cut and are rather thick, not much less than 10 microns, though the difficulties are somewhat less in

this respect since the introduction of what is known as the ' dry method,' but it is still inferior to paraffin for cytological investigations; that they fasten badly to the slides; that the process is unsuited for serial work; and that the celloidin itself stains deeply with aniline dyes.

The *paraffin method* is *par excellence* the method for serial sections, and for all fine and delicate work it is, taking it all round, unrivalled. The paraffin is readily removed from the sections afterwards, and they stick well to the slides. Moreover, thinner sections can be cut by this method than by any other, and the tissues are well preserved. The *disadvantages* are that large objects do not cut well, nor do brittle objects, nor tissues such as fibrous tissue and skin, which have a tendency to become brittle. The whole process requires care and attention both in the preparation before infiltrating, and in the regulation of the temperature during infiltration, if shrinkage and over-heating are to be avoided. But the process gives the finest results to the research student and regular laboratory worker, and is the best general method for really fine sections. Intelligently used, it will carry one triumphantly through apparently insuperable difficulties.

Cutting Sections by the Freezing Method.—Suitable instruments are the Cathcart microtome; the inexpensive students' microtomes made by Jung, Leitz, Bausch and Lomb, etc.; the old-fashioned microtome, having a chamber of ice beneath the plate; and the plate microtome with spray. In the last two the knife is held in a horseshoe three-point suspension tripod, running on adjustable ivory points. One point is at each end of the knife, the other in front, and before starting to cut, the knife must be adjusted so as to have its edge horizontal with the plate, after which sections are cut

seriatim by a slight adjustment of the front screw only. The other microtomes have an adjustable object-carrier, which simplifies matters, and enables truer sections to be cut. Each microtome must, of course, be fitted with ether bottle, bellows, tubing, and the necessary ether points. It will be found that the latter have a tendency to clog, so that a spare pair of points is advisable if one's work is not at times to be disastrously interrupted. For the Cathcart type of microtome a plane-iron in a solid wooden handle is better than a razor. Some of the Continental students' microtomes require special knives.

The object for freezing should be of a convenient size, say $\frac{3}{8}$ inch. It can be frozen without previous preparation, and this is very useful for work that needs to be done in haste; but crystals of ice form in the tissues and distort them, whilst the sections themselves are less satisfactory. Formaldehyde has been used with considerable success in this rapid section-cutting, but it is better to prepare the object in the following simple way: Make a solution of gum mucilage (B.P.) by dissolving 4 ounces of best gum acacia in 6 ounces of water, or use similar proportions. Prepare also a syrup by dissolving a pound of loaf sugar in a pint of water and boiling. This is Mr. Cole's formula. Place the object for twenty-four hours in a mixture of 5 parts of the gum mucilage to 3 parts of the syrup. Take the object from this solution, and remove the excess of solution from its surfaces with a cloth; put a little of the gum mucilage alone on the freezing-plate, and set the ether bellows going; place the object on the frozen mucilage upon the plate, and with a camel-hair brush surround it with mucilage also. It will then be soaked with the mucilage and syrup, but surrounded with mucilage only. Work the bellows until the whole is frozen, and then start

cutting sections, readjusting the knife or object-carrier after each cut, removing the sections from the knife with the brush, and then transferring them to a small shallow glass vessel of distilled water close at hand. If the freezing is carried too far, they will cut badly, and splinter or fly off; in that case stop the ether for a few moments. If the freezing is insufficient, the object will curl up, and perhaps shift. A little practice will soon make perfect, but the following hints may be of service. It may be difficult to freeze sections in a warm room. It will be necessary to add more mucilage from time to time. The blade must be kept cold, or the sections will stick to it. The sections sometimes roll if the saturating mixture contains too little syrup, in which case the tissues must be re-saturated accordingly. Better sections are cut generally when they are done rapidly. Very thin sections cannot be cut by this method, say, thinner than 15 or 20 microns. Sections that have been hardened in alcohol must have the alcohol removed by soaking in alcohol and water, of graduated strengths, say, 50 and 30 per cent., and then washing for a day in running water. Objects can, of course, be stained in bulk before cutting, or the sections can be stained afterwards. If stained in aqueous media they can be mounted at once in glycerine jelly or Farrant's solution, or they can be passed through graduated alcohols and a clearing agent for mounting in Canada balsam.

Cutting Sections by the Celloidin Method.—The relative advantages and disadvantages of the celloidin method have already been discussed. No heat is required throughout the process, nor is it necessary to clear the objects beforehand. Little attention is required, but against this must be put the fact that the procedure is generally a lengthy one, as will be seen later. Indeed,

it can in most of its stages be prolonged indefinitely, which may at times be even a convenience; but three days is a fair average time required.

Celloidin is really a patent preparation of collodion, which is a solution of gun-cotton in ether and strong alcohol. Collodion may be used if necessary, as it is cheaper; but it is better to use celloidin, and it may be bought ready for use in solution, or more generally in horny semi-transparent cubes, of which those supplied by Schering or Grübler are the most trustworthy. It must be cut into thin shreds, and as old celloidin gets very hard, it is well not to keep it too long. As it contains a certain amount of moisture the shavings must be well dried in the open air. They are then dissolved in equal parts of alcohol and ether, which must be what is known as 'sulphuric ether,' and free from acid. Elsching recommends that the celloidin be dissolved independently in alcohol and well stirred, and then an equal quantity of ether added to the solution twenty-four hours later and again stirred, at least another twenty-four hours being allowed to elapse before the solution is used. It must be shaken frequently to keep the heavier part from settling to the bottom. Two solutions will be required: one, known as the 'thin solution,' containing about 5 parts of celloidin by weight to 50 parts of absolute alcohol and 50 parts of ether by measure (5 per cent. solution); and the other, known as the 'thick solution,' containing about 10 parts of celloidin by weight to 50 parts of absolute alcohol and 50 parts of ether by measure (10 per cent. solution).

Objects to be embedded should preferably be about 2 to 8 millimetres thick; the smaller the better. They must be fixed, washed, and *thoroughly* dehydrated in accordance with the principles already laid down on

p. 238, by means of graduated alcohols, finishing with twenty-four hours in 95 per cent. alcohol, and then transferred to absolute alcohol for another twelve to twenty-four hours, with preferably at least one change of alcohol. They must then be transferred to a mixture of absolute alcohol and ether in equal parts for yet another twelve to twenty-four hours, and then put first into the thin celloidin and then into the thick celloidin, so as to get a gradual infiltration throughout. The time required in these celloidin solutions varies within very wide limits; it is never less than twenty-four hours, and often requires some days, and for really large sections weeks or even months may be necessary. But, generally speaking, for objects of the size mentioned, twenty-four to thirty-six hours in each solution is sufficient, the thin celloidin requiring a rather longer time than the thick celloidin. The object will then be ready to affix to the support upon which it is to be cut in the microtome.

The support may be made of wood, glass, or vulcanite, but cork is to be avoided, as it lacks sufficient rigidity. Small wooden blocks a little larger than the object are, perhaps, as easy to obtain as any, though vulcanite is very useful. The wooden blocks should be rectangular and cut across the grain. The end of the block must be dipped into the ether-alcohol solution for a minute or so, then into the thick celloidin solution, and then dried. This is partly to give a hold to the object, and partly to prevent air-bubbles finding their way from the wood into the celloidin mass when the object is fixed in position.

The object may now be removed from the thick celloidin and pressed lightly on the dry surface of the block, or *vice versâ*. It may be necessary to add a little celloidin around it to insure its firmer support. A better way is to run the thick celloidin into a mould

made out of a tiny paper box; to utilize the well-known L-shaped metal embedding blocks; or to use a *dry* watch-glass. A simple and efficient method is to smear a strip of stiff note-paper with a thin film of vaseline, and to wrap it, vaselined side inwards, around the end of the prepared block, tying it on with cotton-thread, and thus forming a neat little cup of suitable size *in situ*. A little thick celloidin is poured into the cup, the object is transferred into it with a needle or forceps and held in the required position, whilst more thick celloidin is poured around it until the cup is full.

The next process is the hardening of the celloidin mass. When a film has formed on its surface, or when it shows just a slight indentation with the ball of the finger (not with the nail), the whole block is put into a small jar, or desiccator, containing chloroform. It can be put bodily into chloroform, though it need not be covered with it; but it is better to utilize the vapour only by using a few drops of chloroform at the bottom of the receptacle and keeping the same carefully closed with a plate, chloroform, of course, evaporating very rapidly, and slow evaporation being advantageous. Eighty-five per cent. alcohol or benzene can be used instead of chloroform, but the process is slower. The alcohol bottle should not be tightly closed. The time required for the hardening will be from one to three hours, though a day or more may be necessary. After this the object is to be transferred to 70 to 85 per cent. alcohol, and here it may remain indefinitely without further attention until required. Sometimes bubbles form in the celloidin, but an hour or two in ether vapour will generally rectify this; and sometimes the celloidin becomes opaque when treated with chloroform, but it becomes transparent again after a time.

The microtome for celloidin sections must be a good one, and one of the Schanze type is best. The knife must be heavy and long, and with a perfect edge, and it should be set obliquely so as to give as large a cutting surface (what is generally mis-described as a 'slicing-cut') as possible. The edge of the object should be set parallel with the knife edge. After each cut the knife and section must be flooded with 70 per cent. alcohol, and if the feed is not automatic, the knife must be lifted clear of the object for each return stroke. Apáthy recommends smearing the knife with vaseline. The sections as they are cut can be transferred with a soft brush, spatula, or glass rod to a watch-glass full of 50, 70, or 95 per cent. alcohol, according to subsequent requirements.

The object can, of course, be stained in bulk before embedding, but otherwise the staining is now to be proceeded with. This is a very important part of the subject, which is dealt with later, but it may be said here that stains fall for the most part into two classes—those which are soluble in alcohol, and those which are soluble in water. Sections for alcoholic stains can be stained at once direct from the watch-glass of 70 per cent. alcohol, but it is not advisable to transfer thin sections direct from strong alcohol to water, as they are liable to become badly distorted. Therefore for watery stains the dehydration process already alluded to must be reversed, and the sections put for three to five minutes into 50 per cent. and then into 35 per cent. alcohol or methylated spirit. Borax-carmine and hæmatoxylin, made according to Delafield's formula, are typical watery stains; the former would require an immersion of half an hour to twelve or twenty-four hours, according to the section, as it is a very non-penetrating stain, whilst ten to thirty minutes would probably suffice for the latter. Alcoholic

eosin would require thirty seconds to a minute. Basic aniline dyes should be avoided for celloidin sections, as far as possible, as the celloidin itself stains rather markedly, though staining of the celloidin may be partly removed by slightly acid alcohol or ether. After staining with a watery stain the sections must be carefully dehydrated by a few minutes in graduated alcohols of 30, 50, and 70 per cent.

If acid alcohol has been used, as suggested above, it must be neutralized by floating the sections first into 70 per cent. alcohol, made faintly alkaline, with a few drops of 0·01 per cent. sodium bicarbonate. This will also restore the blue colour of the hæmatoxylin stain, and make it more permanent. Too much soda will make the colour somewhat diffuse.

Whether the staining has been interpolated or not, the last stage is to transfer the sections from 70 to 95 per cent. alcohol for two to five minutes. Absolute alcohol must not be used, as it dissolves celloidin. The sections are now cleared in carbol-xylol, cedar oil, creasote, or other clearing agent for fifteen to thirty minutes, and mounted in Canada balsam.

When sections are mounted in glycerine the dehydration is, of course, reversed, as for watery stains, and celloidin is quite transparent in this medium.

One mould can be used for several objects, and they can be cut and trimmed after they are set. The ends of the blocks can be softened in ether-alcohol, and the object can then be dipped in thick celloidin and fastened to the block, as described above, by pressing the two together, and then hardening in chloroform or 80 per cent. alcohol.

Sections can be fastened to the slide (which must be clean and dry) by pressing upon them with tissue-paper,

or the albumen method, so much used for serial sections, and hereafter to be described under paraffin embedding, which is more suitable for serial sections, can be made use of. Another method is to flood the sections on the slide with 95 per cent. alcohol two or three times, drain off, follow on with a small amount of ether-alcohol, watch the sections carefully, and as soon as their edges begin to soften expose them to air for thirty seconds, and then immerse at once in 95 per cent. alcohol.

When objects have been stained in bulk they can be cleared also in bulk, say in cedar oil, after the hardening in chloroform, then cut and mounted direct, but the knife must now be flooded with cedar oil instead of alcohol. The final soaking, however, in 95 per cent. alcohol, which is the last stage of dehydration before clearing and mounting, must not be omitted, but in this case it will precede the hardening and clearing instead of following them. This applies particularly to objects which have been kept for some time in 70 per cent. alcohol. The sections and object must not be allowed to dry. Lee recommends a modification of this method —*i.e.*, that after the hardening in chloroform the mass should be put into a mixture of 1 part of chloroform to 1 or 2 parts of cedar oil, and that from time to time more cedar oil should be added so as to bring the mixture gradually up to pure cedar oil. As soon as the object is cleared throughout, the mass may be exposed to the air, and the rest of the chloroform will evaporate gradually. The object may now be mounted on the microtome-holder with a drop of thick celloidin, and either cut at once or kept indefinitely without change in a stoppered bottle. It may be cut dry if done within a few hours, and the cutting quality is often improved by allowing evaporation in the air for some hours. It is better to begin

the hardening, however, with pure chloroform rather than with the chloroform and cedar oil mixture, whilst pure cedar oil is much slower than the latter. Lee strongly recommends these methods, as being much quicker than the older method, giving a more transparent mass, and enabling the object to be cut dry.

Gilson's rapid process (see Lee's ' Microtomist's Vade-Mecum ') much shortens the preliminary stage of embedding. The object is dehydrated as usual, then saturated with ether instead of ether and chloroform, and put into a test-tube containing the thin celloidin solution. The lower end of the tube is dipped into a paraffin bath of melted paraffin, and the celloidin allowed to boil at this low temperature (which does not harm the tissues) until it has been reduced to about a third of its volume and is of the consistency of thick syrup. The mass is then turned out, mounted on a block in the ordinary way, or on a block of hardened celloidin, and the whole hardened for about an hour in chloroform, or in a mixture of chloroform and cedar oil. If chloroform only has been used it is then cleared in cedar oil, which is not necessary if the mixture has been used. It is now cut in the usual way, using cedar oil to flood the knife. The warm bath shortens the process to an hour or so for small objects instead of some days, and the hardening is also proportionately reduced in time.

Scholz also recommends a rapid acetone method in which the fresh tissue is fixed in formalin or alcohol, then put into acetone for thirty minutes to an hour in an oven at 37° C., then put into thin celloidin for four to five hours, then into thick celloidin for two to three hours, maintaining the same temperature throughout, hardened in chloroform for twelve hours or more,

transferred to 70 per cent. alcohol for some hours, and then cut. The rest of the mounting process is as before.

Stepanow's method is to make a solution of 15 grams of celloidin, 50 c.c. of oil of cloves, 200 c.c. of ether, and 10 c.c. of absolute alcohol. The hardened specimen is then transferred from strong alcohol to a small portion of the solution in a closed bottle, left there from three to six hours, put into position on the mounting block, and finally placed in pure chloroform, alcohol, or benzole. The proper consistency for section-cutting is secured in two to three hours, after which transfer is made to 80 per cent. alcohol. If the specimen, after sections are cut from it, is to be kept, it is removed from the block, and placed in chloroform.

Cutting Sections by the Paraffin Method.—We have already dealt with the relative advantages of the paraffin method, as compared with the freezing and celloidin methods, and have explained why this method is by far the most generally useful, especially for thin and difficult sections. Paraffin may be used simply as an *embedding* or supporting medium, and this requires but little explanation. The object is fixed and cleared by any of the usual methods, as explained at length previously, and the melted paraffin is run round the tissue into a suitable mould. Of course, the results are but rough and ready, and the sections are satisfactory only in a few cases. The proper method is that known as the *infiltration* method, where the tissues are thoroughly permeated with paraffin.

A good microtome is necessary, and the knife must be in perfect condition, but on all these essentials stress has already been laid. *Thorough* infiltration is, however, of no less importance, and this must always be

borne in mind. Paraffin is best bought from the dealers, and must be of the proper melting-point. For general use it varies between 43° C. and 58° C. Thin or hard sections require a harder paraffin than thick sections, whilst the temperature of the room also modifies the paraffin. In fact, by adjustment of the temperature of the room in the winter months much can be done to bring refractory sections into a satisfactory state. Generally speaking, with a room at about 16° C., paraffin of 45° C. will be advisable for moderately thin sections, whilst for very thin sections, say, of less than 5 μ, a harder paraffin of 48° to 50° C., or more, may be necessary. Serial sections generally require paraffin with a melting-point of 52° C., or more, up to 58° C. Paraffin should not be melted over an open light, or on a very hot plate, but must always be melted over a water-bath, otherwise the melting-point will rise considerably, and it is not generally known that frequently melted paraffin has the same tendency to increase in hardness and become 'crumbly.' It is not advisable to mix two paraffins of different melting-points to get an intermediate paraffin, as is recommended by some writers, as it is better to buy paraffin of the requisite melting-point, and the control of the temperature of the room, where practicable, will be found of much service. Paraffin of about 50° C. melting-point is perhaps the most generally useful, except for rocking microtomes.

The preliminary stages of fixation, dehydration, and clearing have already been dealt with. The dehydration must be done in graduated alcohols up to 95 per cent., say, thirty to sixty minutes, or even some hours, in each, finishing *always* in absolute alcohol before putting into the clearing fluid. The fixative must be very thoroughly washed out, especially osmic acid and chromic salts,

whilst acetic alcohol must be removed with methylated spirit.

The final clearing agent, however, is not a matter of indifference, apart from the peculiarities of various clearing agents alluded to previously. Turpentine and chloroform mix well with paraffin, but the former is apt to damage delicate tissues, and the latter penetrates badly. Benzole and xylol are excellent solvents for paraffin, but the best general clearing agent is cedar oil, which penetrates well, does not make the object brittle, and mixes well with paraffin. Any trace of alcohol must be scrupulously avoided, as it does not mix with paraffin. The object must be left in the clearing fluid until it looks clear and translucent, and this may take from thirty minutes to a couple of hours.

Moulds of some kind are necessary, and these may be small stiff paper boxes, such as are easily made; trays made of paper or cardboard; 'thimbles' made by wrapping a piece of note-paper round the end of a cork, and leaving the requisite depth projecting, or adjustable moulds made of type-metal. These last are very convenient. They are formed of two L-shaped pieces of type-metal, with rectangular edges, and lie by their own weight upon a plate of metal or glass, the surfaces in contact being slightly glycerined beforehand. For small objects, a watch-glass is the most convenient of all, as it enables accurate orientation to be readily made, and after embedding the object is easily cut out in a rectangular block by means of a slightly-warmed penknife or scalpel.

Paraffin infiltration is unfortunately a somewhat lengthy matter, and it will be evident that variations in temperature must be within very narrow limits. Any considerable increase of temperature is most harmful

It is therefore necessary to carry out the process in a water-bath, or water-oven, provided with a gas-burner and regulator, neither of which need necessarily be very costly. A fairly efficient bath can be fitted up out of an old potato-steamer, some test-tubes, and a thermometer, as mentioned by Mr. M. J. Cole, but this would want tedious watching for proper infiltration. The simplest and cheapest regulator is made on Page's principle, and consists of a large tube partly filled with mercury, immersed in the water of the bath. The end of the tube is closed with a cork, and through this a smaller tube, with its end cut diagonally, is inserted so that it lies inside the large tube, and in contact with the mercury. The gas enters the larger tube by a side branch, and goes out through the smaller inner tube, the end of which lies in the mercury, but is not quite closed by it. A little adjustment for the requisite temperature, measured by a thermometer in the water-bath, is necessary, and then as the mercury expands with any rise in temperature it will more or less close the end of the inner tube, and so reduce the amount of gas which is fed to the burner. These regulators may, if working well, be left unattended for many hours without the temperature of the water-bath varying by more than a degree or two.

The transference of the object from the clearing agent to the melted paraffin may be done gradually, as, for instance, by using an intermediate mixture of paraffin and xylol in equal proportions; but, generally speaking, unless there is a disproportionate amount of clearing agent, it is quite sufficient to transfer the tissues at once from the clearing agent (draining off any excess) to pure paraffin, which has been slowly melted beforehand. The paraffin is then kept melted in the water-bath, at the

requisite temperature, for a time varying from an hour to several days, until it is thoroughly infiltrated. Mr. Lee has a very good working rule by which to judge the time. He gives rather longer than was required to clear the object in the clearing agent. Two hours is a fair average, but a longer time than is necessary is apt to damage the tissues. The paraffin should be kept covered to avoid water-vapour as well as dust. With cedar oil as clearing agent, a change of paraffin is often necessary, and sometimes two changes, but xylol seldom requires any change. If the cedar oil is not thoroughly got rid of, the sections have an annoying tendency to drop out when they are being cut. Very transparent objects may need to be stained before infiltration or to be faintly coloured with a temporary stain, such as Bordeaux red, to enable them to be seen in order to orientate them.

When the tissue is thoroughly infiltrated, the paraffin must be poured into one of the paper boxes, or other moulds already referred to, the object being then transferred, and held in position by warmed needles or fine forceps. As soon as the paraffin shows signs of cooling by becoming opaque, it must be floated at once on to cold water, as it is of importance that it should cool rapidly, or it will crystallize in large instead of small crystals, and will not section so well. Air-bubbles may be a great nuisance, and the rapid cooling also tends to prevent their formation. The water must not come into actual contact with the paraffin itself.

When quite dry and hard the masses of paraffin are removed from their moulds and cut into suitable blocks. These blocks should be as rectangular as possible, and the object should lie near the surface to be cut. For serial sections the upper and lower sides must be truly parallel when in the microtome, otherwise the ribbon of sections

will not run true. When affixed to wooden holders it is advisable slightly to broaden the base of the block. In other than serial sections it is sometimes advisable to make the block somewhat wedge-shaped, so that the narrower end comes first in contact with the knife. They can then be clamped in the microtome, but the best way is to affix them to wooden squares of suitable size. It is only necessary to dip the ends of the wooden squares beforehand into melted paraffin, and then, when required, to attach the paraffin blocks by slightly melting the opposed ends with a warmed knife-blade.

Objects can, of course, be preserved indefinitely in paraffin, and it is easy to scratch upon the block the necessary particulars. The actual cutting of the sections is a comparatively simple matter if all the preliminary details have been well and carefully carried out. Much depends on the edge of the knife or razor, and it must be confessed that the keeping of the edge in first-rate order is not an easy matter, whilst honing and stropping require no little practice. The stropping in particular needs to be done frequently.

The hones are best got from the makers of the microtome, and the best forms are known as yellow Belgian and Blue Water hones, having, of necessity, a very fine grain. The Belgian hone cuts most rapidly, and is most suitable for the preliminary sharpening; whilst the final edge is best obtained on a Blue Water or Arkansas hone. The hones must be of ample surface—say 8 or 9 inches long by 2 inches wide; and as they are rather soft, they must be kept for the one purpose only, and scalpels, knives, or ordinary razors must never be sharpened upon them. A good hone will cost from 12s. upwards. The best lubricant is palm-oil soap made into a lather with water, and after use the oil

must always be carefully wiped from the stone with a soft rag, or it will soak in and harden the stone so as to make it unfit for use. The knife should lie flat upon the stone, concave side downwards, and for large knives their own weight is quite sufficient pressure. The motion should always be in a straight line from left to right, and from heel to toe, the edge being towards the direction of movement, and no circular movement is permissible, whilst the knife must, of course, on no account be sloped upon, much less turned upon, its edge. It is often a tedious operation to put an edge upon a dull or notched knife, but any attempt at hurrying the process only makes matters worse. If a 'paper edge' is formed, a little honing on the other or plane side may be necessary. An examination of the edge under a pocket lens, or even under the microscope, is often of service. The knife must have no notches in it, and must cut easily a hair from the head.

The strop is best obtained from the makers of the knife, and must also be of ample size, but, above all, it must be taut. The less paste there is upon the strop for microtome knives, the better, and the knife must always be stropped in the direction of the grain. Many strops are marked accordingly.

The accompanying illustration shows types of different knives (Fig. 120). The Henking knife has only a short blade, and is comparatively inexpensive; the Jung knife fits into a special clamp, and has a stropping handle; the Weigert and Thoma knives are specially designed to work at an angle, and are expensive, according to length of blade. The first two are the more easily sharpened, but each knife has its own holder to facilitate this, and to insure as far as possible uniformity of angle. The Jung knife is becoming increasingly

popular, not only because it is not so expensive as the Weigert and Thoma knives, but because it can be easily adjusted for tilt, etc., different portions of the blade can be readily used, and it is easier to sharpen. The section of the knife varies according to the class of work required of it, as shown in the illustration (Fig. 121).

The knife can be set square with the object to be cut, or diagonally to it. The former is necessary for

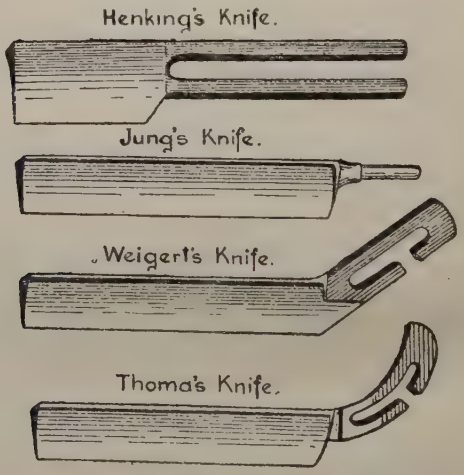


FIG. 120.—TYPES OF MICROTOME KNIVES.

cutting serial sections, and, as already explained, the paraffin block must in this case be carefully trimmed so as to have two of its sides parallel, and to have these sides parallel also to the edge of the knife, otherwise the ribbon will run off crookedly if it runs at all. The knife, when set square, has a greater tendency to crush the sections. When set diagonally, it is more suitable for difficult and uncertain objects, especially those which are large or very soft, or of varying consistency.

The diagonal knife does not, of course, make actually a slicing cut—this is impossible—but by its position it reduces the angle of the section of the knife. For the diagonally-set knife the object should have its sharpest angle, and not its edge, against the knife.

The position of the knife can be varied in yet another way—namely, by slightly altering its tilt—in other words, by altering the relative height of the back and

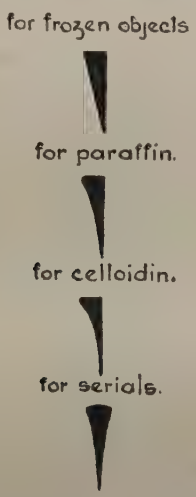


FIG. 121.—SECTIONS OF BLADES OF MICROTOME KNIVES.

edge. The tilt is of some importance, but if excessive, it is apt to break the sections, and is also more liable to make them roll. It should be just enough to enable the edge to clear the section comfortably without scraping or vibration, and is generally rather less for hard than for soft objects. Serial sections in paraffin require but little tilt, whilst celloidin requires a fair amount.

It may be advisable to add here that the adherence of serial sections is facilitated if the trimmed block of hard paraffin is dipped lightly into soft paraffin before cutting, so that the soft paraffin may assist the edges to adhere as they are cut.

The actual cutting of sections has been already dealt with, but a few hints as to common difficulties with paraffin may be of service. Thin sections and those for serials require a rather hard paraffin, as already mentioned, and the temperature of the laboratory must be taken into account. If the paraffin has been overheated in the embedding process, not only is there a danger of spoiling the object, but it will cut badly, and the sections will have a tendency to break and crumble. Such breaking and crumbling is also caused by insufficient embedding, or by the paraffin having failed to displace the clearing fluid. Crushing or wrinkling of the sections is due to a too soft paraffin, a dull knife, a layer of paraffin under the edge of the knife, or to insufficient tilt. A paraffin which is too soft may be cooled in water, or in winter it may be taken into a cooler room. Failing this, the only resource is to cut thicker sections, or to re-embed. Rolling of the sections is generally due to too hard a paraffin, and can often be obviated by very slightly warming the knife, by cutting in a warmer room, by reducing the thickness of the sections, or, finally, by re-embedding. A ribbon of sections not infrequently rolls just at first, but once it has started it goes on all right, especially as the knife or paraffin warms with its work. Serial sections are generally best cut rapidly. A little judicious humouring with a camel-hair pencil will often prove of service. Sometimes rolling is caused by excessive tilt, as already mentioned, whilst a dull knife may have the same tendency. An

obliquely-set knife is also more apt to make sections roll than is a square-set knife.

A longitudinal split or score in the section is due to a piece of grit, or to a notch in the knife edge. Varying thickness of the sections is due to a worn or faulty microtome, or to excessive hardness of the object, or, again, to wrongly adjusted tilt, or even to some play in the object-holder or knife due to insufficient clamping, etc. A hollow-ground razor may cause unequal thickness of the sections, especially with somewhat hard or firm objects, owing to slight spring upon the blade. An annoying occurrence is the sticking of the sections to the knife, either because too soft a paraffin is used, or because of some electric condition of the atmosphere. This may be obviated or diminished by breathing upon the knife whilst the sections are being cut, moisture, of course, assisting in the discharge of the electrical conditions.

Affixing Sections to Slide.—Many sections will require flattening before being attached to the slide or cover-glass. This can be done by placing them in a flat basin of distilled water over a small flame, and regulating the heat so as not to exceed about 37° C. The slide or cover-glass is then dipped beneath the section, and the latter held in place by a needle whilst the slide is gently lifted out. The excess of water is drained off, and the remainder removed by gentle pressure with filter-paper. Blotting-paper should not be used, as it is apt to leave fibres on the sections. The drying can be completed by half an hour on a hot bath at about 35° C., after which the paraffin can be removed by increasing the heat cautiously, cooling, and then immersing the whole slide in xylol, or some other solvent. Sections can be stained in bulk, before cutting or after cutting, but are

best stained after fixation to the cover-glass or slide, as follows:

All slides and cover-glasses must be scrupulously clean, and even if new, are best soaked in one of the strong mineral acids, then washed well with alcohol and put into methylated spirit, where it is well to keep them until required, when they can be removed and dried with a soft cloth. Touching with the fingers must be avoided. A strong hot solution of Hudson's soap is nearly as efficacious as acid. If the slide is not perfectly clean, it will be found that water does not lie evenly upon it, and this is fatal, to serial sections at least. To a perfectly clean slide such as this most sections will adhere if treated with water in the manner just mentioned, or the process may be varied by putting streaks of water on the slide with a sable brush, and then allowing the water partly to drain off or be mopped up, partly to evaporate, but it is essential that the sections should be thoroughly dry before the paraffin is removed either by heat, xylol, turpentine, or other solvent. But adherence of the sections to the slide can be ensured under all subsequent procedures of clearing, staining, etc., by means of a thin film of albumen fixative. Mayer's albumen is made easily, as follows:

White of a new-laid egg	10 c.c.
Pure glycerine	10 c.c.
Sodium salicylate	0·2 gram.

These ingredients must be shaken well together, kept in this condition for a week, and then filtered, which may take another week, and during the process the solution must, of course, be protected from dust. It keeps a long time, and even so small a quantity as the above will be found ample for a great many slides.

There are several ways of using this fixative, but the simplest, quickest, and perhaps the best is that usually adopted in laboratories. A clean, dry slide is taken, and a minute drop of the fixative smeared over it with the ball of a clean finger, and then rubbed away by means of another finger until it is nearly invisible. The slide is now covered with a film of water, the sections duly arranged on it, and the whole then warmed to about 40° C. The warming is best done on a warm plate of metal or glass, a good plan being to get a strip of copper about 8 inches long and 2 inches or so wide, and to support it on an iron tripod with a lamp below, so that one end of the copper only is warmed by the lamp, and the other projects beyond the tripod. When a drop of water placed about the middle of the strip begins to boil, the far end will generally be about warm enough for the slide or cover-glass to be placed on it, and the sections will immediately begin to flatten themselves out. It is important not to raise the heat higher, otherwise the paraffin will melt, even though this may not be apparent, and in the subsequent blotting the sections will stick to the filter-paper. The excess of water can then be drained off, the sections pressed down with filter-paper moistened with alcohol, and then thoroughly dried, in the manner already explained, by means of a hot-air bath, or by keeping the slide at the end of the copper slip, and adjusting the temperature so as not to exceed about 40° C. The author uses the mounting-table shown on p. 246. In about a quarter of an hour the sections should be quite dry, and a few moments longer at a rather higher temperature will melt the paraffin around them, and it can then be removed entirely by immersing the slide bodily in xylol or turpentine. Xylol is the best for this purpose, as liquids

such as benzole and chloroform are rather volatile. For rapid work the drying may be omitted, and the process shortened by waiting a few moments after blotting, then warming the slide enough just to melt the paraffin, and cooling quickly. In many cases this gives quite good results.

The sections can now be stained; and this brings us to the final branch of our subject. It may be noted, however, that one defect of the albumen method of affixing sections is that, thin though the film be, it is apt to stain somewhat with certain stains, such as eosin.

(*c*) Staining.

We have now to deal with a most important part of our procedure—namely, the staining of objects, whether general or differential, in order that their structure may be made more evident. The staining which merely colours a section, or portions of a section, so as to make a pretty picture of it, is of little importance; what is of great importance is that staining which aids the worker by enabling him to differentiate the structures he is endeavouring to study, independently of the resolving power of his lens. It must be remembered that staining is not easy work, and that what seems to be a well-stained preparation may really be quite otherwise. A nucleus, for instance, must not merely be coloured as such, it must show fine structure and not blurred outlines, and if stained for the purpose, should show chromosomes and spindles clearly, and perhaps centrosomes. It is scarcely necessary to insist upon the importance of the matter, and there is no doubt that much of our future advance in microscopical investigation depends on the elaboration of existing staining methods and the discovery of new ones. At the same time we are

nowadays, perhaps, apt to depend too much on the colouring of structures and to neglect careful microscopical examination.

Though much remains to be done, so much has already been accomplished that it is quite impossible to attempt in these pages to describe methods of staining applicable to individual cases. Histology, pathology, bacteriology, clinical methods, zoology, and botany all have volumes devoted to their special and individual requirements, and it is only possible here to give a general *outline* of the broad principles which underlie staining methods in the hope that it may serve as an introduction to their more special applications.

Classification of Stains.—Of course, an efficient stain necessitates a chemical combination between some of its constituents and the section to be stained, but, unfortunately, a chemical classification is impossible in the present state of our knowledge, and any attempt at it would be highly technical and of little value to the ordinary worker. It may be pointed out, however, that a stain may be a *general* stain—that is, it will stain the whole section more or less uniformly of one colour, or it may be a *specific* stain, which means that it has a special affinity for a certain kind or kinds of structure, and that it stains these more than, or to the exclusion of, other structures in the same section. The value of the latter type of stain is obvious.

There are, moreover, two ways of using such *specific* stains. There is the *progressive* method, which selects a suitable specific stain and allows this stain to act until the special structure which it is desired to observe is sufficiently deeply stained to differentiate it from the surrounding structures (which may or may not be themselves stained by the same reagent in a less degree),

and there is the *regressive* method which deliberately overstains, with a view to extracting some of the colour afterwards, leaving the special structures more deeply stained than the others. It may be observed in passing that this 'backward' method is the most generally useful, and is the means by which some of the most useful work has been done.

Attention has already been directed to the great importance of properly 'fixing' an object as one of the first of the necessary steps in its preparation, and it may now be added that objects which are not fixed generally stain in an unsatisfactory manner, whilst objects which are still living practically do not take a stain at all. An exception to this is found, however, in certain stains which are used in very dilute form to stain faintly cells which are yet living. But, as a matter of general practice, all objects must be properly fixed when fresh, and then hardened. Most objects will also be dehydrated, but it is worth noting that the general idea that microscopical material can be kept indefinitely in alcohol until required needs qualification, as long preservation in alcohol frequently militates against subsequent methods of staining.

Before staining, many objects, such as vegetable sections, may require *bleaching*. Steeping in alcohol will generally have this effect, but a solution of chlorinated soda is an excellent bleaching agent without being too powerful. It is made as follows: 1 ounce of dry bleaching-powder is dissolved in a $\frac{1}{2}$-pint tumbler of water, 2 ounces of washing soda are dissolved in another tumbler of water, after which the two solutions are mixed together, well shaken, and allowed to settle for twenty-four hours or so. The clear fluid is then decanted carefully, filtered, and preserved in a stoppered

bottle away from the light. Sections or objects to be bleached are first soaked in water, and then transferred to a small quantity of the chlorinated soda for a period of time varying from one to a dozen hours or more. They must afterwards be very thoroughly soaked or washed in several changes of clean water until every trace of soda is removed.

There are still one or two other considerations. Objects can be stained in bulk before section-cutting, or the sections can be stained after cutting, either loose or affixed to a slide or a cover-glass, as already explained in detail. The staining in bulk is mostly used for *general* staining only, whilst *specific* staining is generally confined to sections, and may then be either progressive or regressive, as mentioned above.

Again, most stains are dissolved in one of two media —either alcohol or water. This is an important distinction, as sections which have been carefully dehydrated would naturally be stained for choice in an alcoholic stain, whilst sections containing water would be most conveniently stained with an aqueous solution. Of course, dehydration or its converse can be carried out previously or subsequently as required, but not infrequently the choice of a stain will depend upon such considerations, or *vice versâ*. Very strong alcoholic solutions of stains are, however, not often used, the final dehydration being completed after staining.

In some cases stains require to be fixed by what are known to calico printers, etc., as ' mordants,' and this may be a deliberate part of the process, and, therefore, of practical importance, or it may be inherent in the method and of more theoretical interest.

Some writers on the subject of staining make classifications such as histological stains and cytological

stains, nuclear stains, plasma stains, etc., but we may with advantage leave these distinctions to text-books dealing with special methods. It is, however, of distinct advantage to realize at an early stage that certain stains have a peculiar affinity for nuclear substance—a special action upon the 'nuclei' of cells—whilst others have an equal affinity for the other elements of cells, known as the 'plasma.'

The chemistry of the subject must be dismissed very shortly, accepting the general classification into 'basic,' 'acid,' and 'neutral' stains. This does not mean that the stains themselves are acid, basic, or neutral; it means that of the chemical components of the stain that part which stains would be, if isolated by chemical means, acid, basic, or neutral, as the case may be. The importance of the distinction to the ordinary worker lies in the fact that 'acid' stains do not wash out in acids, but are made deeper by them; whilst 'basic' stains are intensified by alkalies, and that acid stains are generally more soluble in water than in alcohol, and basic stains more soluble in alcohol. Neutral stains are also more soluble in alcohol.

General Principles.—Before proceeding to deal with a few individual stains in detail it may be advisable to mention certain precautions which must be attended to in order that good and uniform results may be obtained.

1. Formulæ will be given for the preparation of each stain, and these must be carefully followed. The stains must be well filtered before being bottled, and in most cases it is advisable to filter them again just before use, in order to get rid of crystalline products, dust, hairs, etc. A very convenient way of doing the latter is to make of fairly stout wire a small ring about the size of a two-shilling piece, to twist the ends together and bend

them almost at right angles to the ring, to carry the twist down for about 1¼ inches, and then, bending the wire once more, to form with its two ends a rather larger ring immediately beneath the other ring, to serve as a stand. A circular piece of filter-paper, folded into four, is then inserted in the usual way into the upper ring, and through this the stain is filtered direct as required. The portability and size of this little filter-stand make it very convenient for filtering stains direct on to the cover-glass or slide in requisite quantity and without splashing. With regard to the keeping of stains it must be borne in mind that some stains, such as hæmatoxylin, are all the better for being kept, whilst others deteriorate, and some may decompose or even putrefy. The latter can generally be obviated by adding a crystal of thymol or a little salicylic acid.

2. The staining powers of a stain may be increased by using a stronger solution, by allowing it to act for a longer time, by warming the stain, or by adding certain other substances to it—*e.g.*, in some cases, aniline oil.

3. It is a mistake to use too small an amount of stain. There should always be ample to cover the object thoroughly, and often more than this is requisite.

4. The object should be transferred to the stain from a reagent in which the percentage of alcohol or water is nearly the same as that in the stain, and the same applies to the reagent into which it is put immediately after staining.

5. Unsatisfactory staining may be due to the fixative not having been properly washed out, to bad fixation, to the stain being unsuitable for use after certain fixatives, or to an ill-made stain.

In the selection of the stains which follow no principle has been adopted other than that of general usefulness

and comparative ease of application, and those most generally known are naturally chosen. It must be remembered that they are given as examples only, and that no attempt is made to provide anything like an exhaustive list.

Selected Stains.

Carmine.—Let us take this first, though it is less used now than formerly. It is a bright red stain made from the bodies of the cochineal insects. Its active principle is carminic acid. It is most used for *general* staining of entire objects, or of tissues *in bulk* before section-cutting, and for vegetable sections. It is of wide application and easy to use, and the choice of fixing agent is not of importance. There are many formulæ, but Mr. Lee recommends for beginners *Grenacher's alcoholic borax-carmine*, *carmalum*, and *alum-carmine*, and as this selection cannot well be improved upon, these three and *picro-carmine* will be given as examples:

Grenacher's Alcoholic Borax-Carmine.—This is the best solution for vegetable sections and for staining in bulk. Dissolve 1 gram of carmine in 100 c.c. of 4 per cent. borax solution by heating for half an hour or until the carmine dissolves, add an equal volume of 70 per cent. alcohol, allow to stand for twenty-four hours, and filter. Tissues must remain in this stain until thoroughly penetrated, a process which may take some hours or some days, and then be transferred, without washing, into 70 per cent. alcohol, acidulated with a few drops of hydrochloric acid per 100 c.c., and left there until they are differentiated, a process generally taking also a considerable time, at the end of which they will have acquired a more or less transparent appearance. The time required is one of the drawbacks to the use of the stain, and it

will be found to be somewhat erratic both in its staining and penetrating properties on different tissues. On the other hand, the stain is a permanent one. Overstaining may be rectified by means of a weak solution of hydrochloric acid, varying from 0·1 to 1 per cent., but this has a tendency to precipitate the carmine. Dehydrate with 98 per cent. alcohol, clear, and mount in Canada balsam.

Mayer's Carmalum.—Dissolve 1 gram of carminic acid and 10 grams of alum in 200 c.c. of distilled water, with heat. Allow to cool. Filter. Add a few crystals of thymol or 0·1 per cent. salicylic acid as preservative. This stain penetrates tissues in bulk very well, but the process may take anything from a quarter of an hour to several hours. Washing must be done thoroughly in water only if a general plasmatic stain is required, whilst washing with very dilute acid or alum solution will tend to differentiate nuclei. The acid must be thoroughly washed out. Dehydrate gradually with alcohol of increasing strengths—30, 50, 75, and 98 per cent.—clear, and mount in Canada balsam. Carmalum cannot be used as a stain with tissues which are alkaline.

Grenacher's Alum-Carmine.—Both this and the previous stain can be used more particularly for the nervous system. Add ½ to 1 gram of carmine to a 1 to 5 per cent. solution of common alum in 100 c.c. of water. Boil for about a quarter of an hour, cool, and filter. It is a very useful stain for general purposes, as it does not easily overstain, but it does not penetrate well, and is, therefore, less suitable for staining objects in bulk. It has a selective action on nuclei, but must not be used for calcareous structures.

Picro-Carmine.—This is a stain that is less popular now than it used to be, but gives beautiful results with fresh sections. It was formerly supposed to be an

ammonia picro-carminate, but has been shown by Mayer to be an uncombined mixture of carmine, ammonia, picric acid, and other substances in variable and indefinite proportions. As Mr. Lee points out, its *raison d'être* does not lie in its capacity for double staining, as equal or better results can be obtained by staining first with borax-carmine and then after-staining with picric acid, but in the combined stain the picric acid is supposed to neutralize the ammonia. Picro-carmine is, however, useful for fresh tissues, owing to the fixing power of the picric acid. Picric acid stains fibrous tissues and keratin yellow, and acts very rapidly upon them. Bourne's formula for picro-carmine is as follows: Add 5 c.c. of ammonia to 2 grams of carmine in a bottle that will hold about 250 c.c. Stopper, shake, and put aside until next day. Then add slowly, shaking meanwhile, 200 c.c. of a saturated solution of picric acid in distilled water. Put aside for another day. Then filter, add 4 drops of ammonia, and return to the stoppered bottle (Langley). Sections stained in picro-carmine must not be transferred to water, but should be washed in alcohol of not less than 70 per cent. strength, or the surplus stain may be removed by filter-paper and the section mounted in glycerine, or, for a permanent preparation, in Farrant's solution. The staining may take five or ten minutes, or more, and may be hastened by warming the solution. The small amount of stain left in the sections when mounted in glycerine continues to act, and the staining of the section is often much improved after two or three days, and after exposure to daylight. A section of skin stained by this method gives very striking results.

Hæmatoxylin. — This is one of the most generally useful, most dependable, and, therefore, most popular

of stains. It is extracted from logwood, and its active principle is an oxidation product known as ' hæmatein.' It is especially a *nuclear* stain, but may also be used as a plasmatic or general stain, and it gives good results in counterstaining. As these notes are entirely practical, it is not possible to deal at any length with the chemistry of hæmatoxylin staining, so it must suffice to say that hæmatoxylin stains may be made up in a watery or an alcoholic medium, each having its own uses. The stains may be neutral or acid, the neutral stains being more diffuse, whilst the acid stains have a more marked affinity for nuclei, are sharper, and also keep better. Hæmatoxylin is one of those stains which become much less diffuse and more selective by being combined with an appropriate mordant, or by following a fixative or hardening agent which acts as a mordant. The mordants generally used are alum, iron, and chromic acid, especially the first two. Hæmatoxylin follows well after nearly all the ordinary fixatives except, perhaps, osmic acid, which decreases its staining powers.

As hæmatein is an oxidation product, it is necessary that the hæmatoxylin solution should have time to ' ripen,' and this is often a lengthy business, so that it has been recommended that hæmatein itself should be used instead of hæmatoxylin, but these solutions have been found not to keep well, whilst the results have not shown much superiority over the older methods. It has also been suggested that the hæmatoxylin may be ' ripened ' by means of hydrogen peroxide or ammonium carbonate. The ripening of hæmatoxylin is a very uncertain method, and may take several weeks, whilst there is no way to test it save by making a few trials. If kept a long time it may even over-ripen, when it tends to lose its colour, and to deposit a precipitate. Indeed, there

is always some tendency to precipitation, and it is, therefore, advisable to filter the stain immediately before using it. For this purpose the small filter-stand already described (p. 294) answers excellently.

Staining with hæmatoxylin may be either 'progressive' or 'regressive' (see p. 291). The latter gives the best and most sharply differentiated results, and the overstaining is diminished by immersion in $\frac{1}{2}$ to 1 per cent. hydrochloric acid in 70 per cent. alcohol, or by an alum solution of the same strength, subsequent careful washing in water being necessary. For this purpose distilled water should be used, the final washing being done in tap water if 'hard,' or in water containing about 10 grains of bicarbonate of soda to the pint, in order to 'blue' the section. The sections must not be kept indefinitely in water, however, as the colour tends to fade, but must be re-transferred to 70 per cent. alcohol. This final washing free from acid, including that which may be in the stains themselves, must not be neglected.

Alum-Hæmatoxylin Stains.—These are useful stains for objects in bulk, and are used progressively, the alum being in excess and acting as a mordant. It may be noted that celloidin is apt to stain in a rather troublesome way if too little alum be used. Neutral solutions stain blue, whilst acid ones stain red, and in all cases a final wash in tap water or other faintly alkaline water (see above) gives a final deeper blue tinge to the sections. Overstaining can be corrected by weak acid, as previously mentioned, but the acid must be carefully washed out again. Mayer says that turpentine balsam must not be used for mounting. The watery stains keep only indifferently, but the addition of glycerine will enable them to keep some months at least. Glycerine tends also to delay the staining, and so to produce more

uniform results. With age, alum-hæmatoxylin stains tend to become more diffuse, but this can be counteracted by adding a little more alum. The two best-known alum stains are Delafield's and Ehrlich's, which will now be described. Both stains are best used diluted—Delafield's, say, 1 in 10, and Ehrlich's about 1 in 4, and with this strength about twenty minutes on an average will stain most sections satisfactorily.

Delafield's Hæmatoxylin.—This is a *watery* solution, and is made up as follows: Dissolve 1 gram of hæmatoxylin crystals in 10 c.c. of 95 per cent. alcohol. Add it, drop by drop, to 100 c.c. of a saturated aqueous solution of ammonia-alum (say about 1 in 11 of water). Filter, and expose to both light and air for a month or more. Again filter, and then add 25 c.c. of glycerine and 25 c.c. of 95 per cent. alcohol (wood-spirit will do). Set the solution in the light and allow it to stay there until it becomes of a dark purplish colour, then re-filter and keep in a well-stoppered bottle. The solution keeps well and stains rapidly, but should not be used before at least a month has elapsed. It is used rather diluted, and is then more precise. As a watery stain it tends to swell mucus, which it stains well. The water must, of course, be carefully removed by slightly alkaline, graduated alcoholic baths before mounting in Canada balsam.

Ehrlich's Acid Hæmatoxylin.—This is an *alcoholic* solution:

Distilled water	50 c.c.
Absolute alcohol	50 c.c.
Glycerine	50 c.c.
Glacial acetic acid	5 c.c.
Hæmatoxylin	1 gram.
Alum in excess.	

Put this solution into a bottle with a cotton-wool plug only, and allow it to stand in the light for about a month, until it has ripened to a dark claret colour. Then stopper the bottle. It stains rapidly and keeps for years if well stoppered. There is little tendency to over-staining. It is a great favourite for nuclear work, owing to its sharp differentiation of chromatin. The acetic acid is added to prevent precipitation and to enable the stain to differentiate more sharply.

Iron Hæmatoxylin.—This is a rapid and permanent stain, which is of wide and almost universal application. but is especially useful for chromosomes and centrosomes. As Mr. Lee points out, it has a certain optical quality which is peculiarly suited to the employment of high powers. It is also of service for thin sections.

Two solutions are required:

1. Ferric alum	2·5 grams.
Distilled water	100 c.c.
2. Hæmatoxylin	0·5 gram.
Distilled water	100 c.c.

These are not to be mixed, but are to be used separately. The hæmatoxylin solution must ripen as before explained. All alcohol must be removed from the sections by means of water, and they are then placed for from three to six hours or more in Solution 1, rinsed for five minutes in water, then stained for twelve to thirty-six hours in Solution 2, again rinsed with water for a few minutes, and returned to Solution 1 for about ten to thirty minutes, which differentiates the stain. The process should be watched under the microscope, but a change to a dull grey colour is generally a good guide. The sections are finally washed for about an hour in tap water. It is important that all the iron-alum

should be washed out, or the sections will fade later on. If any iron-alum is left in the sections before transference to Solution 2 it will turn the hæmatoxylin black; but this does not matter. The fine differentiation of cell structure may require twelve or more hours in both solutions. The clearing must be done with xylol, and the mounting in xylol or benzole balsam. This stain is mentioned because it is the best of the hæmatoxylin stains, and is well worth trouble in mastering.

Coal-Tar Stains.—We have now to deal with a very important and useful class of stains which are derivatives of coal-tar products, and are known as such, or under the name of aniline dyes. These stains are generally easy to use, and give good results, though the results are not always as permanent as could be wished. They fade very quickly if mounted in glycerine, but keep fairly well in Canada balsam. They combine readily with the tissues, and seldom require the use of a mordant, but most of them give their best results when the tissues are overstained, and the excess of colour is removed. This, as explained previously, is an example of the 'regressive' method of staining, by which sharp differentiation is most easily obtained. Otherwise these stains tend to be somewhat diffuse. The number of coal-tar stains in use is considerable and is growing, but as it is intended here to explain principles rather than detailed methods, only a few of those most commonly in use will be mentioned. Of these, two—methyl green and Bismarck brown—may be looked upon as acting best when allowed to stain *progressively*.

Methyl Green.—A suitable formula would be:

Methyl green	0·5 gram.
Acetic acid	1·0 c.c.
Rectified spirit	20 c.c.
Distilled water	80 c.c.

Stain for about five minutes, rinse thoroughly with slightly acidulated distilled water, if necessary differentiate rapidly with 90 per cent. alcohol, also slightly acidulated, and mount in glycerine, glycerine-jelly, or Farrant's solution. This stain is most useful for fresh tissues, particularly botanical ones, and it attacks chromatin in particular. It is therefore a nuclear stain. Mr. Lee calls attention to its sensitiveness to the action of alkalies, and rightly lays stress upon the necessity of seeing that all solutions for washing or mounting are neutral or faintly acid. It is an advantage if the stain is made up with a little acetic acid, as in the formula given above, and all washing and dehydrating media must also be slightly acidulated. The glycerine or Farrant's solution used as the mounting medium must be made very slightly acid too. The stain acts almost immediately, and is very penetrating, but there is little tendency to overstain. It is not easy to mount in Canada balsam, as the stain comes out very rapidly in the alcohol necessary for dehydration, and in aqueous media, as above, the stain unfortunately fades rather rapidly, so that it is not suitable for really permanent preparations.

This stain is often used with borax-carmine for 'double staining' vegetable sections, which are first stained with methyl green, washed rapidly in water, and then stained in borax-carmine.

Bismarck Brown (Vesuvin).—This is a useful nuclear stain for either fresh or hardened tissues, and is also used as a counterstain for bacteria. It is, perhaps, most conveniently made up as follows:

Bismarck brown	0·5 gram.
Rectified spirit	20 c.c.
Distilled water	80 c.c.

or it may be made up as a saturated aqueous solution for *intra vitam* staining. It is not easily washed out, and is permanent, but stock solutions are best filtered just before use. Like methyl green, it stains quickly, but does not overstain. The stain is used *progressively*, and the tissues may be mounted either in aqueous media such as glycerine, or in Canada balsam.

The stains next to be mentioned are typical *regressive* stains. They may be made up with either water, or alcohol, but are generally used in considerable strength, and the sections must always be *overstained*. The time required may vary from ten minutes up to twenty-four hours. The subsequent extraction of the excess of stain, with its diffuse coloration, is by means of 95 per cent. absolute alcohol, and it is as well just to dip the sections first in water before differentiating. The stain comes out very rapidly in alcohol, at first in streams, and looks as if it would all be extracted, but if the section has been sufficiently stained the excess of colour which is coming out will be found to get less until, in about half a minute to two minutes, it has nearly ceased. Examination will then show that the section has cleared up wonderfully, and that the staining of the selected elements is sharp and precise. A little alcohol in a watch-glass is quite enough to use. Sometimes it is advisable to make the alcohol slightly acid with a drop or two of dilute hydrochloric acid. The sections are subsequently cleared and mounted in Canada balsam, the best clearing agents being cedar oil or xylol. Clove oil extracts yet more colour, and is apt also to distort the tissues. Mr. Lee points out that the results depend largely upon the fixing agent that has been used.

The following *regressive* stains of the coal-tar series are those most commonly used.

Safranin.—This is a most useful stain, and gives excellent and permanent results. It is especially useful for rapidly growing and dividing tissues. It is important to purchase the stain from a reliable maker, such as Grübler. 'Safranin O' is commonly used. Safranin is not easily soluble in water, and is best made up with aniline water, as follows:

Safranin	1 gram.
Aniline water..	90 c.c.
Alcohol, 95 per cent.	10 c.c.

'Aniline water' is made by adding about 5 c.c. of aniline oil to 100 c.c. of distilled water, shaking well, and filtering through a wet filter-paper. The stain must be filtered immediately before use, but it keeps for an indefinite time if kept in the dark. The time required for staining may be as much as twenty-four hours. It stains nuclei a beautiful red, but requires differentiating with alcohol as described above.

Gentian Violet.—This is also a most useful *regressive* stain, which stains nuclei blue. A suitable formula is:

Gentian violet	1 gram.
Alcohol, 95 per cent.	20 c.c.
Aniline water..	80 c.c.

The aniline water is made as above, and is best added at the time of staining only. The stain is a very precise one, and is useful with thin sections, but requires differentiation with alcohol as already described.

Gentian violet is, however, more especially used for staining bacteria, and though special methods of staining are purposely omitted, it is of such general use for this purpose that this particular application may, with ad-

vantage, be described, the more so as it is an excellent example of *selective* methods. The following is the method known everywhere as 'Gram's method' for differentiating bacteria, and it requires, in addition to the above solution, the following:

Iodine	1 gram.
Iodide of potassium	2 grams.
Water	300 c.c.

The bacteria, fixed in the usual way by heat upon the cover-glass, are stained for about five minutes with gentian violet in the cold, or the watch-glass containing the stain can be heated until the steam rises, in which case a minute will suffice. The cover-glass is then washed in water and placed for half a minute to two minutes in the iodine solution. The film will rapidly turn black. It is then transferred to alcohol or methylated spirit, and agitated in this until the film loses colour and becomes a pale grey. The film is then washed in water, dried between filter-paper and in air, and when quite dry, mounted in Canada balsam.

The advantage of this stain is that it selects certain bacteria which *retain* the stain, whilst others entirely lose it, and thus it is of great service clinically, in distinguishing definite pathological bacteria. Amongst those which *retain* the stain are the bacilli of tubercle, leprosy, diphtheria, tetanus, yellow fever, and anthrax, and *Streptococcus*, *Staphylococcus*, *Micrococcus tetragenus*, and *Diplococcus pneumoniæ*. Amongst those which are *decolorized* are the bacteria of bubonic plague, Asiatic cholera, influenza, dysentery, glanders, typhoid, *Bacillus coli communis*, *Diplococcus intracellularis*, *Gonococcus*, and *Micrococcus melitensis*.

An excellent stain for general work is:—

Carbol Fuchsin.—Ziehl's formula is as follows:

Fuchsin	1 gram.
Carbolic acid crystals	5 grams.
Alcohol	10 c.c.
Distilled water	100 c.c.

Differentiate with alcohol as before. This stain is much used in medical work for staining tubercle bacillus, and differentiating is then done with an acid, tubercle being distinguished as an 'acid-fast' bacillus. The film on the cover-glass is put in the stain in a watch-glass and heated until the steam rises, perhaps several times. The film is then washed in water, and decolorized in, say, 20 per cent. solution of sulphuric acid for two or three minutes, which takes out the stain from all other structures except the bacilli. The film is again washed thoroughly in water, dipped for a couple of minutes into alcohol, and mounted in Canada balsam in xylol. If necessary, counterstaining may be done with methylen blue for, say, half a minute, but overstaining of the background must be carefully avoided, as it is important that the bacilli should be very marked.

Amongst the *plasmatic* stains two may be taken as examples, of which the first is:

Picric Acid.—This stain colours fibrin, fibrous tissue, skin, bone, etc., a bright yellow. It acts rapidly, and is very penetrating and permanent. It is useful for staining objects whole, and is much used for small *Arthropoda*, *Nematodes*, etc. It is, of course, a very diffuse stain, and is often employed in combination with other stains, as in Van Gieson's stain, to be next described. In using this stain it is only necessary to add a little picric acid to some alcohol, or to one of the alcohols used for dehydrating, but water must be

avoided, nor should the alcohol, indeed, be of less strength than 70 per cent., as the fixation is thereby affected. Mr. Lee gives the useful reminder that alcoholic solutions to which picric acid is added should be *neutral* and not acid, as the presence of acid greatly increases its power of washing out carmine stains. After carmine stains, therefore, washing in acidulated alcohol is inadvisable, and it is only necessary to rinse, say, for two minutes in neutral alcohol of not less than 70 per cent. strength, and then to stain for a few minutes in picric acid, finally dehydrating in absolute alcohol for three to four minutes and clearing in cedar oil.

Van Gieson's Stain is made up as follows:

Acid fuchsin, 1 per cent. aqueous solution ..	10 c.c.
Picric acid, saturated aqueous solution ..	90 c.c.

This stain is often used with hæmatoxylin for fibrous or nerve tissue. Nuclei and epithelium are stained brown, white fibrous connective tissue red, elastic tissue and muscle yellow. Sections are slightly overstained with hæmatoxylin, washed in water, and then put for, say, five seconds in Van Gieson's stain. The subsequent dehydration and clearing have a tendency to extract the colour given by the picric acid, so that both alcohol and clearing agents, such as xylol, may with advantage have a crystal or two of picric acid added.

Eosin.—This is also a favourite diffuse plasmatic stain, and is largely used for staining ova in embryological work. It is also used as a contrast stain with hæmatoxylin, staining cell-structures red, whilst the nuclei are blue; but sections require careful washing before being transferred from one stain to the other, as eosin precipitates hæmatoxylin. Eosin may be dissolved in water or alcohol, say 0·5 per cent. solution, or even in

the clearing agent. It is a specific stain for red blood-corpuscles, and for granules found in certain of the leucocytes. When used with hæmatoxylin for embryological tissues, sections may be stained for fifteen minutes to an hour with a strong solution of eosin in 90 per cent. alcohol, washed quickly with alcohol, and then stained for twenty minutes or half an hour with a rather weak solution of hæmatoxylin. A favourite use of eosin is to stain blood-films in connection with methylen blue. Jenner's formula is, perhaps, the most useful. Add

Water-soluble eosin (Grübler)	1·25 gram.
Distilled water	100 c.c.
To an equal quantity of methylen blue	1 gram.
Distilled water	100 c.c.

Mix in a basin, and stir well. Allow to stand for twenty-four hours, filter, wash the precipitate well upon the filter with distilled water, dry it thoroughly, take 0·5 gram of dried powder and dissolve in 100 c.c. of pure methyl alcohol, and again filter. The solution keeps well. Blood-films merely require dipping in this for about three minutes, rinsing in distilled water till pink, drying rapidly high over a flame, and mounting in xylol balsam. The making of blood-films is beyond the purpose of this book, but for those who have never attempted it we may mention that a drop of blood may be obtained with a *clean* needle from the finger or the lobe of the ear, and just touched with a cover-glass. A second cover-glass is then immediately placed upon the drop on the first cover-glass, and the two drawn quickly apart, so that they slide over each other to make a thin film. The secret is to use absolutely clean cover-glasses, which have been cleansed in bichromate of potassium, and kept in alcohol till required, and to make the thinnest (almost invisible) film possible. Some

people use the edge of a cigarette paper for this latter purpose, but a little practice enables one to make excellent films by the former method. The film may be dried in the air, but it is better to fix it with *gentle* heat before staining.

Methylen Blue.—The last important stain that will be dealt with is methylen blue, which is also one of the coal-tar compounds. It is of much value in making preparations of the nervous system, and gives good results with intracellular cement-substance, not inferior to impregnation processes with gold chloride and silver nitrate. Its use with eosin in staining blood-corpuscles has just been mentioned. It must not be confused with methyl blue. It is a useful *intra vitam* stain, and is in this respect convenient for the study of small semi-transparent aquatic forms of life, where it is only necessary to colour the water containing the organisms a light blue, and to watch the staining until it is satisfactory, remembering, however, that the organisms readily lose their colour again. Sometimes, after reaching a certain point, they begin to lose colour as readily as they took it up. For nerve-terminals a 1 per cent. solution of methylen blue in normal saline solution (NaCl 0·75 per cent.) is generally used, the tissue being perfectly fresh, and exposed freely to the air during the staining. The process must be examined every few minutes under a microscope, and stopped when the required structures are sufficiently stained. The section must not be allowed to dry, but must, on the other hand, not be thickly covered. The stain is very unstable, however, unless the tissue is immediately transferred to a saturated solution of ammonium picrate and left there for from six to twenty-four hours. The final mounting is done in glycerine, or in equal parts of glycerine and

ammonium picrate, in which the tissues may with advantage soak for twenty-four hours before mounting. For general nuclear staining an ordinary aqueous solution is used, and allowed to stain for one to twenty-four hours, or a solution may be made up as follows:

Methylen blue	..	..	..	0·25 gram.
Rectified spirit	..	..	..	20 c.c.
Distilled water	..	..	..	80 c.c.

To stain nuclei, sections are stained with the above solution for five minutes, rinsed well in distilled water, differentiated in 90 per cent. alcohol, dehydrated, cleared, and mounted in Canada balsam. The washing in distilled water is important, as it fixes the stain better against the decolorizing effect of the subsequent treatment with alcohol.

Silver Nitrate.—This is given as an example of 'impregnation' or metallic deposit methods, of which gold chloride is another well-known example. It is used for epithelium, endothelium, etc. A 0·5 to 1 per cent. solution of silver nitrate in *distilled* water is used, and the section, after washing in distilled water, is placed in this for a few minutes (say two to five) and then rinsed again in distilled water and exposed to sunlight or bright daylight (in alcohol or water, according to whether it is to be mounted in Canada balsam or glycerine), until the stained parts turn brown.

The list of stains given in the preceding pages could, of course, be multiplied indefinitely, but it is hoped that the foregoing will serve as examples of *general principles*, and that definite applications will be sought for in text-books specially devoted to their respective subjects, some of which will be found mentioned in the Bibliography at the end of this book. First of these for

English histologists and zootomists who are doing serious work stands Mr. Bolles Lee's 'Microtomist's Vade-Mecum,' which has been frequently alluded to, and to which the author is indebted for not a few of the statements made in this chapter. For botanical work Strasburger's 'Practical Botany' will be found useful, or Zimmermann's 'Botanical Micro-Technique,' though the latter is perhaps somewhat out of date. For amateur work Cross and Cole's 'Modern Microscopy' may be highly recommended.

Conclusion.—In conclusion, may we earnestly urge upon those readers who do not use the microscope for any definite purpose the necessity of taking up some particular subject of study, and of using the microscope as a means to that end, rather than as an interesting optical toy for idly examining heterogeneous slides, which by themselves will soon lose their novelty and interest? Rightly used, the microscope is a means to an end, rather than an end in itself, and is capable of opening out to its owner ever-widening fields of fascinating and absorbing study and occupation. We cannot all be great scientific discoverers, but we may all be builders of the temple of science, if it be only to lay one single brick in that rapidly-growing structure, or to supply a little of the clay or straw out of which such a brick may be constructed by others.

APPENDIX

It may be helpful if a list is given of the most useful books dealing with the microscope, and with the general animal and plant life for which the microscope is so essential a means of study, to which are also appended short explanatory notes. This list makes no claim to be in any sense a complete bibliography of the subject.

Technology and Applications.

Carpenter, Dr. W. B.: The Microscope and its Revelations (ed. Dr. Dallinger). 21 plates and 800 illus., demy 8vo. *Churchill*, 1901. (The standard work.) 36s.

Hogg, Jabez: Microscope—History, Construction, and Application. 20 plates and 447 illus., cr. 8vo. *Routledge*, 1898. (Ranks next after Carpenter.) 10s. 6d.

Schacht, H.: The Microscope and its Application. (Trans.) Post 8vo. *Churchill*, 1855. (Chiefly vegetable.) 6s.

Wright, Lewis: A Popular Handbook to the Microscope (ed. A. H. Drew). Ill., cr. 8vo. *Religious Tract Society*, 1922. (An excellent book for beginners.) 5s.

Technology.

Bagshaw, W.: Elementary Photo-Micrography. 3rd ed. Cr. 8vo. *Iliffe*, 1915. 1s.

Barnard, J. E.: Practical Photo-Micrography. 20 plates, 79 illus., demy 8vo. *Arnold*, 1911. (Complete.) 15s.

Bausch, E.: Manipulation of the Microscope. Ill., post 8vo. *Bausch & Lomb Co.* (For beginners.) $1.

Beale, Dr. L. S.: How to Work with the Microscope. 600 illus., cr. 8vo. *Harrison*, 1886. (Rather out of date.) 21s.

BECK, CONRAD: Cantor Lectures on the Theory of the Microscope. 74 illus. *Royal Society of Arts*, 1908. 1s.

BECK, CONRAD: The Microscope. [Part I.] A Simple Handbook. Cr. 8vo. *Beck*, 1921-24. (Practical.) 2s. 6d.

BECK, CONRAD: The Microscope. Part II. An Advanced Handbook. Cr. 8vo. *Beck*, 1924. (Deals mainly with optics.) 7s. 6d.

BOUSFIELD, Dr. E. C.: Guide to the Science of Photo-Micrography. Ill., cr. 8vo. *Churchill*, 1892. (A thoroughly practical book.) 6s.

CHAMBERLAIN, C. J.: Methods in Plant Histology. Ill., demy 8vo. *Chicago*, 1906. (Practical exercises.)

CLARK, C. H.: Practical Methods in Microscopy. 8vo. *Boston*, 1896. $1.60.

COLE, A. C.: Critical Microscopy. Demy 8vo. *Churchill*, 1921. (Rather scrappy.) 7s. 6d.

CROSS, M. I., and COLE, M. J.: Modern Microscopy. 5th ed. an. H. F. Angus. Ill., demy 8vo. *Baillière, Tindall & Cox*, 1922. (An excellent book. Deals with the use of the microscope and mounting.) 10s. 6d.

DAVIES, T.: Preparation and Mounting of Microscopic Objects. Ill., 12mo. *C. A. Pearson*, 1905. (Fair. A reprint of an old edition.) 1s.

DAVIS, G. E.: Practical Microscopy. 310 illus. and coloured front., 8vo. *W. H. Allen*, 1895. (Useful to beginners, but rather out of date.) 7s. 6d.

DOWDY, S. E.: The Microscope. How to Choose and Use It. *Soman*, 1921. 5s. 6d.

EYRE, J. W. H.: Elements of Bacteriological Technique. 170 illus., demy 8vo. *W. B. Saunders & Co.*, 1913. (For laboratory work only.) 16s.

FLATTERS, A.: Methods in Microscopical Research: Vegetable Histology. 23 coloured plates, sm. 4to. *Sherratt & Hughes*, 1905. (A series of elementary lessons, and atlas of elementary botanical sections.) 21s.

FREY, Prof. H.: Technology of the Microscope. (Trans.) Ill., cr. 8vo. *New York*, 1880. $6.

FRIEDLÄNDER, Prof. C.: Microscopical Technology. (Trans.) Ill., 16mo. *New York*, 1886. $1.

GAGE, S. H.: The Microscope. 12th ed. 200 illus., demy 8vo. *New York*, 1920. (An introduction to microscopical methods. The most recent and well-known American book.) $1.50.

GUYER, M. F.: Animal Micrology. 74 illus., demy 8vo. *Chicago*, 1907. (Practical exercises in human histology and in zoology.) 9s.

HALL, C. A.: How to Use the Microscope. A Guide for the Novice. *Black*, 1924. 2s. 6d.

HALL, I. W., and HERXHEIMER, G.: Methods of Morbid Histology and Clinical Pathology. Demy 8vo. *W. Green & Sons*, 1905. (For medical laboratory work.) 9s.

HIND and RANDLE. Handbook of Photo-Micrography. Cr. 8vo. *Routledge*, 1914. 10s. 6d.

HEATH, C. E.: Beginner's Guide to the Microscope. *Percival Marshall & Co.*, 1922. 1s. 6d.

JAMES, F. L.: Elementary Microscopical Technology. 8vo. *St. Louis*, 1887. (A manual for students.) $0.75.

LEE, A. B.: The Microtomist's Vade-Mecum. 8th ed. Demy 8vo. *Churchill*, 1921. (Standard work on advanced histological methods.) 28s.

MALLEY, A. C.: Micro-Photography; Wet Collodion, Gelatino-Bromide Process. Crown 8vo. *H. K. Lewis*, 1885. 7s. 6d.

MALLORY AND WRIGHT: Pathological Technique. 8th ed. 156 illus., demy 8vo. *W. B. Saunders & Co.*, 1924. (For medical laboratory work.) 32s. 6d.

MANN, G.: Physiological Histology: Methods and Theory. Demy 8vo. *Clarendon Press*, 1902. (Theoretical rather than practical.) 15s.

MARSH, S.: Microscopical Section-Cutting. Ill., 12mo. *Churchill*, 1882. (Chiefly animal.) 3s. 6d.

MARTIN, J. H.: Manual of Microscopic Mounting. Ill., 8vo. *Churchill*, 1878. (Chiefly medical.) 7s. 6d.

MCJUNKIN, F. A.: Clinical Microscopy and Chemistry. 21s.

MELL, P. H.: Biological Laboratory Methods. 124 illus., cr. 8vo. *Macmillan & Co.*, 1902. (Useful to biologists.)

MILLS: Photography Applied to Microscope. 12mo. *London*, 1891. 2s.

NÄGELI, Prof. C., and SCHWENDENER, Prof. S.: Microscope in Theory and Practice. (Trans.) 2nd ed. 210 illus., 8vo. *Sonnenschein*, 1892. (Chiefly theory.) 21s.

PENDLEBURY, C. Lenses and Systems of Lenses. Cr. 8vo. *Bell*, 1886. (After Gauss.) 5s.

PHIN, J.: How to Use the Microscope. (For beginners.) $1.40.

Practical Hints on the Selection and Use of the Microscope. 8vo. *New York*, 1881.

PRINGLE, A.: Practical Photo-Micrography. 3rd ed. Ill., sm. 4to. *Iliffe*, 1902. (Perhaps the most practical book on the subject.) 3s. 6d.

'QUEKETT CLUB MAN': Handbook of the Microscope. 38 illus., cr. 8vo. *Roper*, 1887. (Selection and management.) 2s. 6d.

QUEKETT, JOHN: Practical Treatise on the Use of the Microscope. *London*, 1855.

SCHNEIDER, A.: Microscopy and Micro-Technique. Ill. *Chicago*, 1899.

SEILER, C.: Compendium of Microscopical Technology. 12mo. *Philadelphia*, 1880. $1.

SPITTA, E. J.: Photo-Micrography. 6 plates and 63 illus., large 4to. *Scientific Press*, 1899. (Excellent plates.) 12s. 6d.

SPITTA, E. J.: Microscopy: Construction, Theory, and Use of Microscope. 3rd ed. 241 illus. and 47 half-tone reproductions of photo-micrographs. Demy 8vo. *Murray*, 1920. (Deals lucidly with theory and manipulation.) 25s.

SQUIRE, P. W.: Methods and Formulæ used in the Preparation of Animal and Vegetable Tissues for Microscopical Examination. Post 8vo. *Churchill*, 1892. (Standard book.) 3s. 6d.

STOKES, A. C.: Microscopy for Beginners. Ill., cr. 8vo. *New York*, 1887. $1.50.

STRANGEWAYS, T. S. P.: Clinical Pathology and Practical Morbid Histology. 3rd ed. Cr. 8vo. *Strangeways*, 1903. (Class-book for medical students.) 5s.

VAN HEURCK, Dr. H.: The Microscope: its Construction, Manipulation, and Technique. (Trans.) 4to. *Lockwood*, 1893. 18s.

WALMSLEY, W. H.: The A.B.C. of Photo-Micrography. *New York*, 1903. $1.25.

WEST, G.: The Practical Principles of Plain Photo-Micrography. *Campbell*, 1916. 4s. 6d.

WHITE, T. C.: The Microscope, and How to Use It. Ill., fcp. 8vo. *Sutton.* (Elementary mounting methods.) 3s.

WINSLOW, C. E. A.: Elements of Applied Microscopy. Ill., cr. 8vo. *Chapman & Hall*, 1905.

WRIGHT, Sir A. E.: Principles of Microscopy. Coloured plates, large demy 8vo. *Constable*, 1906. (Mainly theory.) 25s.

ZIMMERMANN, A.: Botanical Micro-Technique. Demy 8vo. *Constable*, 1896. 12s.

APPLICATIONS.

ASH, E. C.: Pond Life. 1s.

BEHRENS, Prof. H.: A Manual of Micro-Chemical Analysis. (Trans.) Ill., cr. 8vo. *Macmillan*, 1900. (A well-known book.) 6s.

BEHRENS, W. J.: The Microscope in Botany. (Trans.) Ill., 8vo. *Boston*, 1885. $5.

BUTLER, E. A.: Pond Life Insects. (Young Collector Series.) 8vo. 1886. 1s. 6d.

CLARKE, L. LANE: Objects for the Microscope. 8 coloured plates, cr. 8vo. *Groombridge*, 1871.

CLAYTON, E. G.: A Compendium of Food-Microscopy: 282 illus., demy 8vo. *Baillière, Tindall & Cox*, 1909. 12s.

COLE, A. C.: Methods of Microscopical Research. 2nd ed. 1895.

COLE, A. C.: Diseases of the Blood. 10s. 6d.

COLE, H. C. (ed.): Studies in Microscopic Science. Coloured plates, 8vo. *Baillière*, 1883-86. 4 vols. Vol. I., 27s. 6d.; Vols. II., III., IV., each, 31s. 6d.

COOKE, M. C.: 1,000 Objects for the Microscope. Ill., cr. 8vo. *Warne*, 1900. (For amateurs.) 2s. 6d.

DANIELS AND NEWHAM: Laboratory Studies in Tropical Medicine. 5th ed. 1923. 25s.

FURNEAUX, W.: Life in Ponds and Streams. 8 coloured plates and 331 figs., cr. 8vo. *Longmans*, 1897. 6s.

GALT, H.: Microscopy of Starches. Ill., sm. 8vo. *Baillière, Tindall & Cox*, 1900. 3s. 6d.

GOSSE, P. H.: Evenings at the Microscope. Ill., cr. 8vo. *S.P.C.K.*, 1895. (Elementary. A delightfully written book.) 4s.

GREAVES AND WRIGHTON: Practical Microscopical Metallography. *Chapman and Hall*, 1925. 16s.

GREENISH, H. G.: Microscopical Examination of Food and Drugs. 3rd ed. 168 illus., large 8vo. *J. and A. Churchill*, 1923. 10s. 6d.

GREENISH, H. G.: Anatomical Atlas of Vegetable Powders. 138 illus., large 8vo. *J. and A. Churchill*, 1904. 12s. 6d.

GRIFFITH, J. W., and HENFREY, A.: Micrographic Dictionary (ed. Rev. M. J. Berkeley and Prof. T. R. Jones). 15 plates and 818 illus., representing 2,680 figs., 8vo. *Gurney*. 1883. (A standard work of reference.) 52s. 6d.

HANAUSEK, Dr. T. F.: Microscopy of Technical Products. 276 illus., large 8vo. *Chapman and Hall*, 1907. 21s.

Handbook of Instructions for Collectors. Ill., post 8vo. *Trustees of British Museum*, 1902.

HARKER, A.: Petrology for Students. 6th ed. Post 8vo. *Cambridge University Press*, 1923.

HATCH, T. H.: Text-Book of Petrology. 6th ed. Post 8vo. 1910. 7s. 6d.

HOWE, H. M.: Iron, Steel, and other Alloys. Cr. 8vo. 1906. 30s.

JOHANNSEN, A. Manual of Petrographic Methods. *New York*, 1914.

KERR, R.: Hidden Beauties of Nature. Ill., 8vo. *Religious Tract Society*, 1900. 2s. 6d.

KERR, R.: Nature through Microscope and Camera. 65 plates, demy 8vo. *Religious Tract Society*, 1905. (Photo-micrographs, with descriptions.) 6s.

KERR, R.: Nature's Curiosity Shop. Post 8vo. 1922.

LANGLEY, J. N.: Practical Physiology. Ill., cr. 8vo. *Macmillan*, 1920. (For students of elementary physiology.) 10s. 6d.

LANKESTER, E.: Half-Hours with the Microscope. Ill., 12mo. *C. A. Pearson*, 1905. (A reprint of an old edition.) 1s.

LEACH, A. E.: Food Inspection and Analysis. 4th ed. 40 plates and 120 figs., large 8vo. *Chapman & Hall*, 1920. 31s. 6d.

MARSHALL, C. E.: Microbiology. 3rd ed. 1924. 21s.

MARTIN, J. H.: Microscopic Objects Figured and Described. 194 illus., 8vo. *Van Voorst*, 1871. 14s.

MINOT, C. S.: A Laboratory Textbook of Embryology. 2nd ed. Ill. *Philadelphia*, 1911. (Methods for embryological work.)

MUIR AND RITCHIE: Manual of Bacteriology. 8th ed. 1921. 16s.

OERTEL, E. T.: Medical Microscopy. *Rochester, N.Y.*, 1903. $2.

OSMOND, F.: Microscopic Analysis of Metals. *Griffin*, 1925. 10s. 6d.

PARKER, T. J. and W. N.: Practical Zoology. 5th ed. Ill., post 8vo. *Macmillan*, 1920. (For students of elementary zoology.) 15s.

PREGL, F.: Quantitative Organic Micro-Analysis. *Blakiston*, 1924. 12s. 6d.

PRIMROSE AND SEXTON: Practical Metallography of Iron and Steel. 2nd ed. Cr. 8vo. *Manchester*, 1923. 4s.

RHODES, J. E. W.: Micro-Petrology for Beginners. 1912. 3s. 6d.

'QUEKETT CLUB MAN': My Microscope and Some Objects from my Cabinet. 5 illus., fcap. 8vo. *Roper*, 1888. 2s. 6d.

SAUVEUR, A.: The Metallography of Iron and Steel. 1918. $6.

SCHAEFER, E. A.: Essentials of Histology. 11th ed. 553 illus., cr. 8vo. *Longmans, Green & Co.*, 1920. (Standard work for medical students.) 14s.

SCHERREN, H.: Ponds and Rock-Pools. Ill., cr. 8vo. *Religious Tract Society*, 1894. (Good.) 2s. 6d.

SCHERREN, H.: Through a Pocket Lens. Ill., cr. 8vo. *Religious Tract Society*, 1897. 2s. 6d.

SCHNEIDER, A.: Micro-Biology and Micro-Analysis of Foods. 1921. 21s.

SHELLEY, H. C.: Chats about the Microscope. Ill., post 8vo. *Scientific Press*, 1899. 2s.

SLACK, H. J.: Marvels of Pond-Life. Ill., cr. 8vo. *O. Newman & Co.*, 1892. (Well-known book.)

SPIERS: Nature through the Microscope. 10 coloured plates and 300 illus. *Culley*, n.d.

STRASBURGER, E.: Handbook of Practical Botany. 8th ed. (Trans.) 149 illus., cr. 8vo. *Sonnenschein*, 1920. (Well-known book.) 12s. 6d.

WALLIS, T. E.: Analytical Microscopy. Post 8vo. *Arnold*, 1923. 6s.

WETHERED, A.: Medical Microscopy. Ill. *London*, 1892.

WHIPPLE, G. C.: The Microscopy of Drinking-Water. 3rd ed. 19 plates, large 8vo. *Chapman & Hall*, 1914. 15s.

WINTON, A. L.: Microscopy of Vegetable Foods. 2nd ed. 589 illus., large 8vo. *Chapman & Hall*, 1916. 31s. 6d.

WOOD, Rev. J. G.: Common Objects of the Microscope. Ill., cr. 8vo. *Routledge*, 1900. (Reprint of well-known book.) 2s. 6d.

PERIODICALS.

ANNUAL OF MICROSCOPY. *Lund Humphries.* 1899-1900. Each, 2s. 6d. (Two numbers alone published.)

JOURNAL OF APPLIED MICROSCOPY, monthly. *New York*, 1898-1903. (Discontinued.) 4d.

JOURNAL OF THE ROYAL MICROSCOPICAL SOCIETY, every two months. *Williams & Norgate.* 6s. (Contains original papers and abstracts of research done in all branches of microscopical science throughout the world, and records all advances in microscopy.)

JOURNAL OF THE QUEKETT MICROSCOPICAL CLUB, every six months. *Williams & Norgate.* 3s. 6d.

KNOWLEDGE, monthly. 27, *Chancery Lane, W.C.* (Gave special attention to microscopy. Discontinued.) 1s.

QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE, not strictly quarterly. *Churchill.* (Original papers, advanced.) 10s.

Some of the foregoing books are out of print. In every case when the date is given it is the most recent known to the author. Only books or periodicals written in or translated into the English language are included.

APERTURE TABLE.

(From the Royal Microscopical Society's Journal.)

Numerical Aperture. ($n \sin u = a$.)	Corresponding Angle ($2u$) for—		
	Air ($n=1{\cdot}00$).	Water ($n=1{\cdot}33$).	Homogeneous Immersion ($n=1{\cdot}52$).
1·52	..	..	180° 0′
1·50	..	..	161° 23′
1·45	..	..	145° 6′
1·40	..	..	134° 10′
1·35	..	..	125° 18′
1·33	..	180° 0′	122° 6′
1·30	..	155° 38′	117° 35′
1·26	..	142° 39′	111° 59′
1·24	..	137° 36′	109° 20′
1·20	..	128° 55′	104° 15′
1·10	..	111° 36′	92° 43′
1·00	180° 0′	97° 31′	82° 17′
0·98	157° 2′	94° 56′	80° 17′
0·96	147° 29′	92° 24′	78° 20′
0·94	140° 6′	89° 56′	76° 24′
0·92	133° 51′	87° 32′	74° 30′
0·90	128° 19′	85° 10′	72° 36′
0·88	123° 17′	82° 51′	70° 44′
0·86	118° 38′	80° 34′	68° 54′
0·84	114° 17′	78° 20′	67° 6′
0·82	110° 10′	76° 8′	65° 18′
0·80	106° 16′	73° 58′	63° 31′
0·78	102° 31′	71° 49′	61° 45′
0·76	98° 56′	69° 42′	60° 0′
0·74	95° 28′	67° 37′	58° 16′
0·72	92° 6′	65° 32′	56° 32′
0·70	88° 51′	63° 31′	54° 50′
0·68	85° 41′	61° 30′	53° 9′
0·66	82° 36′	59° 30′	51° 28′
0·64	79° 36′	57° 31′	49° 48′
0·62	76° 38′	55° 34′	48° 9′
0·60	73° 44′	53° 38′	46° 30′

APERTURE TABLE (*Continued*).

Numerical Aperture. ($n \sin u = a$.)	Corresponding Angle ($2u$) for—		
	Air ($n = 1{\cdot}00$).	Water ($n = 1{\cdot}33$).	Homogeneous Immersion ($n = 1{\cdot}52$).
0·58	70° 54′	51° 42′	44° 51′
0·56	68° 6′	49° 48′	43° 14′
0·54	65° 22′	47° 54′	41° 37′
0·52	62° 40′	46° 2′	40° 0′
0·50	60° 0′	44° 10′	38° 24′
0·45	53° 30′	39° 33′	34° 27′
0·40	47° 9′	35° 0′	30° 31′
0·35	40° 58′	30° 30′	26° 38′
0·30	34° 56′	26° 4′	22° 46′
0·25	28° 58′	21° 40′	18° 56′
0·20	23° 4′	17° 18′	15° 7′
0·15	17° 14′	12° 58′	11° 19′
0·10	11° 29′	8° 38′	7° 34′
0·05	5° 44′	4° 18′	3° 46′

USEFUL MEMORANDA

MEASURES AND WEIGHTS.

1 millimetre = ·03937 inch.

The unit used in microscopy is the *micron*, generally written μ = ·001 millimetre. The thousandth part of a micron is written $\mu\mu$.

One inch = 25·3999 millimetres.
,, = 25·4 millimetres approximately.
One foot = 30·4799 centimetres.
,, = 30·48 centimetres approximately.
One gram = 1 cubic centimetre of distilled water at 4° C.
,, = 15·432 grains.
One grain = ·064792 gram.
One ounce avoirdupois = 437·5 grains = 28·349 grams.
,, ,, apothecaries = 480 grains = 31·103 grams.
One kilogram = 2½ lbs. avoirdupois approximately.
One cubic centimetre = 16·9 minims = $\frac{1}{16}$ cubic inch approximately.
One minim = ·05916 c.c.
One fluid drachm = 3·5495 c.c.
One fluid ounce = 28·396 c.c.
One pint = 567·92 c.c.
One quart = 1·1358 litres.
One litre = 35·2154 fluid ozs.

TEMPERATURES.

To convert Centigrade into Fahrenheit $\left(C \times \frac{9}{5}\right) + 32 = F.$

,, ,, Fahrenheit into Centigrade $(F - 32) \times \frac{5}{9} = C.$

STANDARD "SOCIETY" GAUGES.

Screw Thread for Objectives.

Form.—Whitworth—*i.e.*, **V**-shaped thread, angle 55°, one-sixth of **V** depth rounded off at top and bottom.

Pitch.—Thirty-six threads per inch. Length of thread 0·125 inch (3·175 millimetres).

Full diameter ..	..	max. 0·7982 in. (20·274 mm.)
,, ,, ..	..	min. 0·7952 ,, (20·198 mm.)
Effective diameter	..	max. 0·7804 ,, (19·822 mm.)
Core ,,	..	,, 0·7626 ,, (19·370 mm.)

Screw Thread for Nosepiece.

Form, pitch, etc., as above.

Full diameter ..	..	min. 0·8000 in. (20·320 mm.)
Effective diameter	..	,, 0·7822 ,, (19·868 ,,)
Core ,,	..	,, 0·7644 ,, (19·416 ,,)
,, ,,	..	max. 0·7674 ,, (19·492 ,,)

Diameter of Draw-Tubes for Eyepieces and Sub-Stage.

	Minimum.	*Maximum.*
Small	0·917 in. (23·299 mm.)	0·918 in. (23·325 mm.)
Large	1·270 ins. (32·258 ,,)	1·271 ins. (32·283 ,,)
Extra large	1·410 ,, (35·814 ,,)	1·411 ,, (35·839 ,,)
Sub-stage	1·527 ,, (38·786 ,,)	1·529 ,, (38·937 ,,)

The fit of the eyepieces and sub-stage condenser fitting is left to the manufacturers, but their diameter must not exceed the minimum diameter given above.

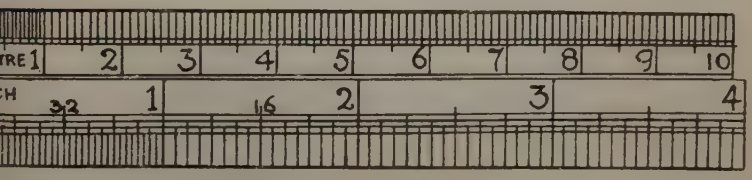


Fig. 122.—Centimetre and Inch Scale.

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